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Editorial

Yawning as a clinical clue? Revisiting COMISA phenotypes in sleep-disordered breathing

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Keywords: obstructive sleep apnea, sleep bruxism, COMISA, yawning

Nocturnal yawning may represent a subtle clinical marker of arousal instability in patients with sleep-disordered breathing (SDB), potentially unveiling latent comorbid insomnia and sleep apnea (COMISA) phenotypes, and expanding the interpretative scope of sleep-related behaviors.

The recent case report by Michałek et al. describes a rare and intriguing co-occurrence of yawning during sleep in a patient with obstructive sleep apnea (OSA) and sleep bruxism (SB).[1] The researchers' hypothesis of a hypoxia-triggered arousal mechanism is compelling and well-grounded in the observed polysomnographic patterns. Nonetheless, it would be interesting to integrate further reflections that may expand and enrich the interpretation of this complex behavioral constellation.

One critical angle that deserves consideration is the possible presence of insomnia-related physiology, particularly within the framework of comorbid insomnia and sleep apnea (COMISA). Although this diagnosis has not been formally addressed, some reported features, such as marked sleep fragmentation, microarousals and low adherence to positive airway pressure (PAP) therapy, are often part of some COMISA phenotypes and may reflect a background of sleep-state hyperarousal, which is frequently observed in those patients.[2] Indeed, it has been recently proposed that COMISA encompasses a heterogeneous spectrum, with emerging interest in identifying clinical phenotypes.[2]

While such phenotypic models are still in the hypothesis-building stage and require stronger empirical validation, the existence of subclinical insomnia manifested through subtle neurophysiological and behavioral mechanisms, even in the absence of subjective complaints, is increasingly recognized as a relevant issue in both clinical practice and sleep research.[3] In this context, the observed nocturnal yawning might be not only a direct response to hypoxia, but also a behavioral expression of the underlying arousal instability, which could reflect a latent insomniac phenotype with challenging aspects from the clinical point of view. This possibility highlights an often-overlooked dimension in sleep medicine – the presence of non-overt insomnia traits that nonetheless modulate sleep architecture, treatment adherence and the emergence of sleep-related motor behaviors.[4] Moreover, COMISA patients frequently show the dysregulation of sleep–wake transitions, a state that can facilitate

the intrusion of transitional behaviors, such as yawning, into otherwise consolidated sleep.[5] Also, recent evidence suggests that SB may play a mechanistic and phenotypic role in COMISA, potentially reflecting a hyperarousal-driven motor expression that is intensified in the presence of overlapping insomnia and OSA. In a polysomnographic study by Blaszczyk et al., patients with COMISA presented a significantly higher frequency of rhythmic masticatory muscle activity (RMMA) and bruxism-related arousals as compared to patients with OSA alone.[5] These findings support the idea that SB may serve as a physiological link between cortical arousal and autonomic dysregulation, helping to explain the heightened motor instability observed in COMISA cases. Yawning, traditionally associated with sleep onset or state transitions, may in these cases re-emerge inappropriately due to an incomplete inhibition of wake-promoting networks, possibly exacerbated by the repeated respiratory-related arousals of OSA, eventually associated with motor exacerbations. Similar mechanisms were described in clinical reports of unusual yawning patterns associated with SB and OSA.[6]

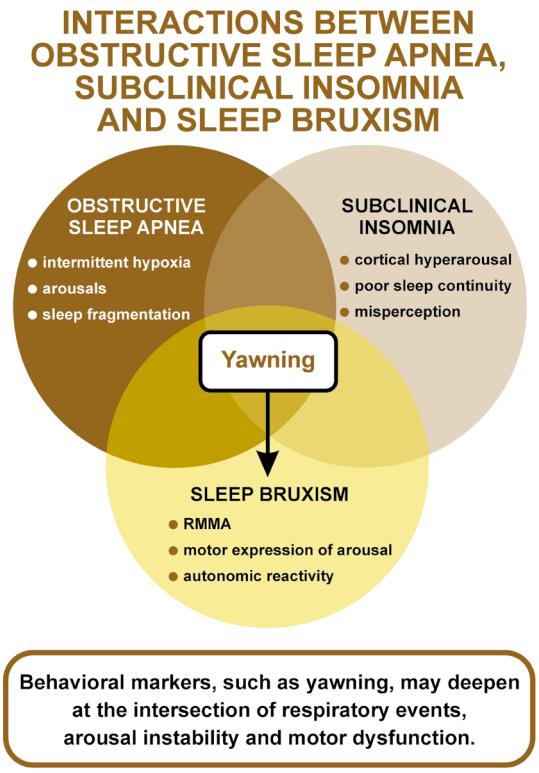

Fig. 1. Interactions between obstructive sleep apnea (OSA), subclinical insomnia and sleep bruxism (SB) – hypothesized pathways leading to nocturnal yawning as a behavioral marker

RMMA – rhythmic masticatory muscle activity.
This conceptual diagram illustrates the proposed overlap between 3 physiological and behavioral domains: OSA (characterized by intermittent hypoxia and respiratory-related arousals); subclinical insomnia (marked by cortical hyperarousal and sleep-state instability); and SB (representing a motor expression of arousal and autonomic reactivity). At the intersection of these mechanisms, yawning may emerge as an atypical yet potentially informative behavioral marker. Though rare, its occurrence could reflect the underlying arousal instability in comorbid insomnia and sleep apnea (COMISA) phenotypes, especially when traditional symptoms are absent or subtle.

This proposal is further strengthened by neurobiological insights. As Blaszczyk et al. noted, yawning, bruxism and sleep fragmentation share overlapping neurochemical pathways, particularly those involving dopaminergic modulation.[5] The hypothesis that bruxism and yawning share common subcortical circuitries, particularly within the basal ganglia and hypothalamus, could reflect a broader state of central disinhibition, where motor pattern generators are more likely to get activated in the presence of disturbed arousal regulation.[7,8] These considerations align with recent data on the etiological mechanisms and metabolic consequences of OSA, including inflammation, oxidative stress and insulin resistance.[9,10]

From a clinical perspective, raising awareness about such subclinical or latent forms of insomnia embedded in OSA presentations is vital. These forms often go unrecognized due to the absence of explicit complaints, but may substantially influence treatment outcomes, including tolerance to PAP therapy, symptom persistence and behavioral adherence. Recognizing this overlap, particularly through unusual motor or autonomic markers, such as nocturnal yawning, may open new diagnostic and therapeutic pathways.

As illustrated in Fig. 1, yawning may emerge at the intersection of OSA, subclinical insomnia and SB, reflecting shared arousal-related mechanisms. Nocturnal yawning might serve as a clinical clue, albeit subtle and unspecific, of latent COMISA physiology, especially in patients who otherwise present a predominantly respiratory sleep disorder phenotype. Such behavioral markers may reflect deeper layers of arousal instability and neurochemical dysregulation that are not easily captured by conventional sleep diagnostics.

In conclusion, the unusual case reported by Michałek et al.[1] provides fertile ground for reflection. While the hypoxia–arousal mechanism is essential, the integration of additional domains, namely subclinical insomnia expression, COMISA-related hyperarousal, neurochemical overlap, and behavioral arousal markers, may offer a broader, more integrative understanding of these phenomena. On the other hand, framing yawning as a potential clinical clue, rather than a mere physiological reflex, invites us to revisit the complexity of COMISA phenotypes and rethink how subtle behaviors during sleep can guide us toward more precise diagnostic and therapeutic strategies.

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References

1. Michałek M, Marschollek K, Kuliczkowski W, et al. An unusual case of yawning during sleep associated with sleep bruxism and obstructive sleep apnea – a case report. *Front Med (Laussanne)*. 2025;12:1596512. doi:10.3389/fmed.2025.1596512
2. Meira e Cruz M, Sweetman A. Comorbid insomnia and sleep apnea: From research to clinical practice. *Semin Respir Crit Care Med*. 2025;46(2):113–124. doi:10.1055/a-2591-5664

3. Sigurdardottir FD, Bertisch SM, Reid ML, et al. Association between insomnia phenotypes and subclinical myocardial injury: The Multi-Ethnic Study of Atherosclerosis. *Sleep*. 2023;46(4):zsac318. doi:10.1093/sleep/zsac318
4. Benjamins JS, Migliorati F, Dekker K, et al. Insomnia heterogeneity: Characteristics to consider for data-driven multivariate subtyping. *Sleep Med Rev*. 2017;36:71–81. doi:10.1016/j.smrv.2016.10.005
5. Blaszczyk B, Meira e Cruz M, Waliszewska-Prosol M, et al. Sleep bruxism and sleep structure in comorbid insomnia and obstructive sleep apnea (COMISA) syndrome: A polysomnographic study. *J Clin Med*. 2024;13(11):3154. doi:10.3390/jcm13113154
6. Wulterkens BM, Hermans LW, Fonseca P, et al. Sleep structure in patients with COMISA compared to OSA and insomnia. *J Clin Sleep Med*. 2023;19(6):1051–1059. doi:10.5664/jcsm.10500
7. Uchima Koecklin KH, Aliaga-Del Castillo A, Li P. The neural substrates of bruxism: Current knowledge and clinical implications. *Front Neurol*. 2024;15:1451183. doi:10.3389/fneur.2024.1451183
8. Doelman CJ, Rijken JA. Yawning and airway physiology: A scoping review and novel hypothesis. *Sleep Breath*. 2022;26(4):1561–1572. doi:10.1007/s11325-022-02565-7
9. Karuga FF, Jaromirska J, Sochal M, Białasiewicz P, Gabryelska A. Association between glucose metabolism, the circadian cycle and hypoxia: Evaluation of the NPAS2 and Rev-Erb-α protein serum levels in obstructive sleep apnea patients – a pilot study. *Dent Med Probl*. 2024;61(3):465–469. doi:10.17219/dmp/185718
10. Kanclerska J, Wieckiewicz M, Nowacki D, et al. Sleep architecture and vitamin D in hypertensives with obstructive sleep apnea: A polysomnographic study. *Dent Med Probl*. 2024;61(1):43–52. doi:10.17219/dmp/172243

Comment

The Postgraduate Orofacial Pain and Dysfunction Program at the Academic Centre for Dentistry Amsterdam: Celebrating two decades of graduates

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Keywords: orofacial pain, bruxism, tooth wear, obstructive sleep apnea, dental education

This paper outlines the development of a distinctive, full-time, 3-year postgraduate Master of Science (MSc) program in Orofacial Pain and Dysfunction (OPD), which integrates advanced clinical training, interdisciplinary care and research publication.

Evolution of a dental discipline

Over the past decades, the dental discipline of orofacial pain and dysfunction (OPD) has evolved globally from a rather narrow, mainly dental domain with a major focus on temporomandibular disorders (TMDs) to a much broader, more medical domain that encompasses not only TMDs, but also other (non-dentoalveolar) types of orofacial pain (including neuropathic pain and headaches), oral movement disorders (including one of their most common and sometimes debilitating consequences, i.e., tooth wear in association with bruxism) and dental sleep disorders (including obstructive sleep apnea (OSA), gastroesophageal reflux (GER) and oral moistening disorders). Due to the multifactorial nature of the numerous conditions that are part of the OPD discipline, some of them partly overlapping with other dental disciplines (e.g., endodontology, oral and maxillofacial surgery) and many of them also being part of several medical disciplines (e.g., neurology, otolaryngology, anesthesiology, rheumatology), OPD requires dentists with specialized knowledge and skills to assess and manage the patients affected by OPD conditions in interdisciplinary settings. In 2020, the National Commission on Recognition of Dental Specialties recognized orofacial pain as the 12th dental specialty in the USA.[1] Notably, also elsewhere in the world, there is an increasing focus on more and better OPD training. This global evolution of the OPD discipline has paved the way for new educational programs.

A new postgraduate OPD program

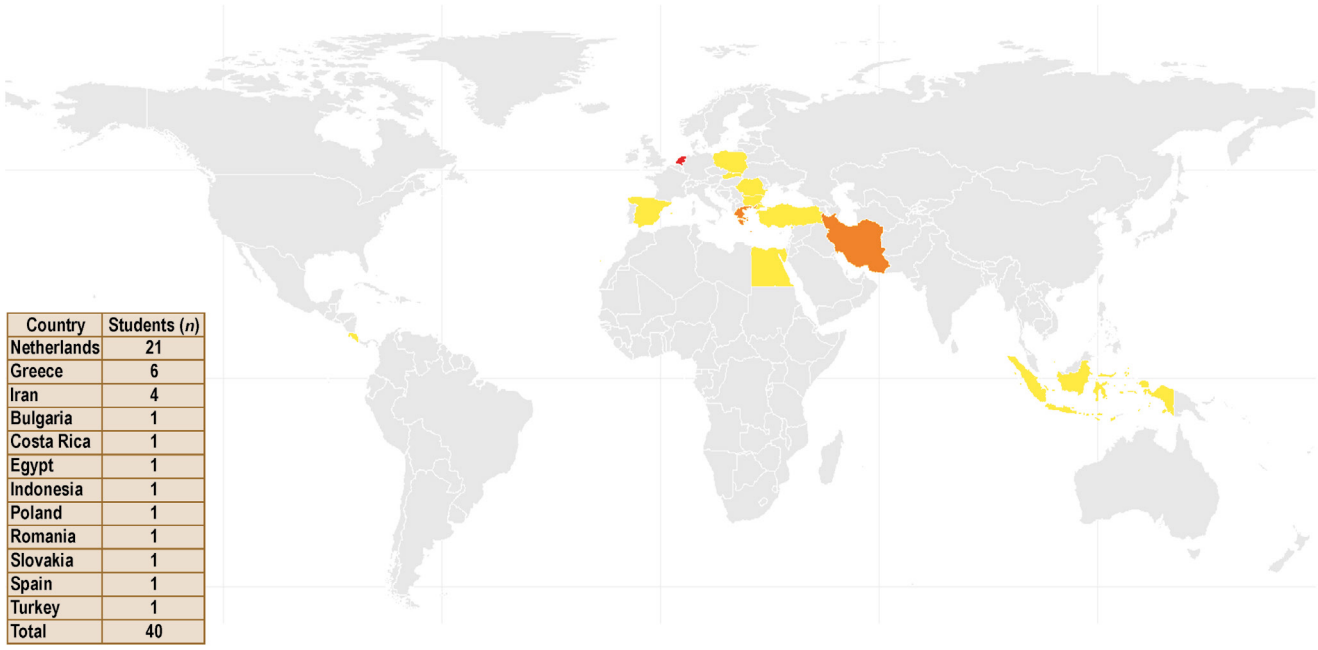
In anticipation of this global evolution, a 3-year postgraduate OPD program was developed at the Academic Centre for Dentistry Amsterdam (ACTA), the Netherlands. In September 2003, the first student was admitted to the program. From the start, the program was officially recognized by the University of Amsterdam as a postgraduate Master of Science (MSc) program. Information on the background and design of this new program was published previously.[2] The program had a director (Prof. Frank Lobbezoo) and a co-director (Dr. Jacques van der Zaag), and attracted students from many different countries – most of them from the Netherlands, but also significant numbers from Greece, other European countries, and beyond (Fig. 1). The program's graduates, the first one finalizing in June 2006, pursued different careers. Some stayed at the Department of Orofacial Pain and Dysfunction of ACTA to obtain their PhD degree and/or to become a teacher/clinician, while others started a career as an orofacial pain specialist at another academic institution, in a center for special dental care or in a private practice, either in the Netherlands or outside. Yet others returned to their home country for the next phase in their careers.

Oral Health Sciences program

Around the time of the start of the OPD program in 2003, ACTA had several other postgraduate programs to offer. Some of them were well-established, like the ones for Periodontology and Endodontology; others, including the OPD program, were more in their infancies, like the ones for Pediatric Dentistry and Implantology/Prosthodontics. All programs had their own design, management and, in some cases, accreditation. It was ACTA's ambition to create a single postgraduate program, with several graduation profiles (i.e., OPD, Periodontology, Endodontology, Pediatric Dentistry, and Implantology/Prosthodontics). The overarching postgraduate program was named Oral Health Sciences (OHS): A 3-year full-time program, taught in English, with 60 European Credits (ECs) per year – hence 180 ECs in total. The postgraduate OHS program started in September 2015. The OPD profile continued with a structure that was comparable to the early days, but now embedded in the larger OHS structure with its additional modules and activities. From the start of the newly structured program, the profile director was Dr. Peter Wetselaar, with Prof. Frank Lobbezoo as the co-director. In 2022, the role of profile director was taken over by Dr. Michail Koutris.

Uniqueness of the program

The OPD profile of the postgraduate OHS program,[3] or the OPD-OHS program in short, is unique for several reasons. It is one of the few full-time programs worldwide that train dentists in all aspects of OPD (supervisors: Dr. Michail Koutris, Prof. Frank Lobbezoo, Dr. Marisol Reyes-Sevilla, and Prof. Corine M. Visscher), including dental sleep disorders (supervisors: Prof. Ghizlane Aarab and Dr. Marisol Reyes-Sevilla) and tooth wear (supervisors: Dr. Ruud H. Kuijs, Dr. Chryssa Papagianni and Dr. Peter Wetselaar). For the latter condition, a specialized clinic was founded at the Department of Orofacial Pain and Dysfunction that focuses on the assessment,



Country	Students (*n*)
Netherlands	21
Greece	6
Iran	4
Bulgaria	1
Costa Rica	1
Egypt	1
Indonesia	1
Poland	1
Romania	1
Slovakia	1
Spain	1
Turkey	1
Total	40

Fig. 1. Heatmap showing the countries from which the postgraduate students of the Orofacial Pain and Dysfunction (OPD) program originated (2003–2025)

management and – when indicated – restorative treatment of the worn dentition, using non- or minimally invasive techniques whenever possible. Also the patients referred with a diagnosis of OSA are treated in a specialized departmental clinic, where, besides counseling, mandibular advancement appliances are offered. And obviously, a large clinic for OPD is part of the department's clinical realm. In that clinic, an interdisciplinary team is active, including orofacial pain specialists (in training), orofacial physical therapists, and health psychologists. In such a rich clinical environment, the postgraduate students are immersed in the complex and interconnected problems of the patients living with OPD conditions, which contributes enormously to the students' development as orofacial pain specialists. Of important notice is the fact that in all parts of the clinic, clinicians work according to the newest insights and guidelines, including those for orofacial pain and TMDs,[4–10] bruxism,[11–15] tooth wear,[16–18] and OSA.[19,20]

Apart from the clinical and educational components of the OPD-OHS program, the postgraduate students are required to perform a research project as part of their training. The project is preferably of such a level that the publication of the outcomes in an international peer-reviewed journal is possible. Indeed, most students manage to have their work published, while all students produce a postgraduate master's thesis that includes a detailed description of their research. In Table 1, some examples of the published works of the postgraduate students of the OPD profile are provided. From the listed co-authors of these publications, the team of supervisors of the various projects can be gathered.

Table 1. Examples of peer-reviewed publications based on the research performed by the postgraduate students (**bold names**) of the Orofacial Pain and Dysfunction (OPD) program*

No.	Publication
1.	**Kalaykova S**, Naeije M, Huddleston Slater JJ, Lobbezoo F. Is condylar position a predictor for functional signs of TMJ hypermobility? *J Oral Rehabil*. 2006;33(5):349–355. doi:10.1111/j.1365-2842.2005.01572.x
2.	**Koutris M**, Lobbezoo F, Naeije M, Wang K, Svensson P, Arendt-Nielsen L, Farina D. Effects of intense chewing exercises on the masticatory sensory-motor system. *J Dent Res*. 2009;88(7):658–662. doi:10.1177/0022034509338573
3.	**Wetselaar P**, Lobbezoo F, Koutris M, Visscher CM, Naeije M. Reliability of an occlusal and nonocclusal tooth wear grading system: Clinical use versus dental cast assessment. *Int J Prosthodont*. 2009;22(4):388–390. PMID:19639077.
4.	**Papagianni CE**, Van der Meulen MJ, Naeije M, Lobbezoo F. Oral health-related quality of life in patients with tooth wear. *J Oral Rehabil*. 2013;40(3):185–190. doi:10.1111/joor.12025
5.	**Osiewicz MA**, Manfredini D, Loster BW, Van Selms MK, Lobbezoo F. Comparison of the outcomes of dynamic/static tests and palpation tests in TMD-pain patients. *J Oral Rehabil*. 2018;45(3):185–190. doi:10.1111/joor.12600
6.	**Van Der Lugt CM**, Rollman A, Naeije M, Lobbezoo F, Visscher CM. Social support in chronic pain: Development and preliminary psychometric assessment of a new instrument. *J Oral Rehabil*. 2012;39(4):270–276. doi:10.1111/j.1365-2842.2011.02269.x
7.	**Nikolopoulou M**, Naeije M, Aarab G, Hamburger HL, Visscher CM, Lobbezoo F. The effect of raising the bite without mandibular protrusion on obstructive sleep apnoea. *J Oral Rehabil*. 2011;38(9):643–647. doi:10.1111/j.1365-2842.2011.02221.x
8.	**Te Veldhuis AH**, Lobbezoo F, **Te Veldhuis EC**, Naeije M, Van Selms MK. Synovial chondromatosis of the temporomandibular joint. A systematic review of the literature on its characteristics [in Dutch]. *Ned Tijdschr Tandheelkd*. 2011;118(9):421–426. doi:10.5177/ntvt.2011.09.10267
9.	Kalaykova SI, **Klitsie AT**, Visscher CM, Naeije M, Lobbezoo F. A retrospective study on possible predictive factors for long-term temporomandibular joint degeneration and impaired mobility in juvenile arthritis patients. *J Oral Facial Pain Headache*. 2017;31(2):165–171. doi:10.11607/ofph.1656
10.	**Marpaung CM**, Kalaykova SI, Lobbezoo F, Naeije M. Validity of functional diagnostic examination for temporomandibular joint disc displacement with reduction. *J Oral Rehabil*. 2014;41(4):243–249. doi:10.1111/joor.12130
11.	**Valiente López M**, Van Selms MK, Van der Zaag J, Hamburger HL, Lobbezoo F. Do sleep hygiene measures and progressive muscle relaxation influence sleep bruxism? Report of a randomised controlled trial. *J Oral Rehabil*. 2015;42(4):259–265. doi:10.1111/joor.12252
12.	**Warnsinck CJ**, Koutris M, Shemesh H, Lobbezoo F. Persistent dento-alveolar pain disorder (PDAP) [in Dutch]. *Ned Tijdschr Tandheelkd*. 2015;122(2):95–100. doi:10.5177/ntvt.2015.02.14189
13.	**Attallah MM**, Visscher CM, Van Selms MK, Lobbezoo F. Is there an association between temporomandibular disorders and playing a musical instrument? A review of literature. *J Oral Rehabil*. 2014;41(7):532–541. doi:10.1111/joor.12166
14.	Lövgren A, **Parvaneh H**, Lobbezoo F, Häggman-Henrikson B, Wänman A, Visscher CM. Diagnostic accuracy of three screening questions (3Q/TMD) in relation to the DC/TMD in a specialized orofacial pain clinic. *Acta Odontol Scand*. 2018;76(6):380–386. doi:10.1080/00016357.2018.1439528
15.	Visscher CM, **Van Wesemael-Suijkerbuijk EA**, Lobbezoo F. Is the experience of pain in patients with temporomandibular disorder associated with the presence of comorbidity? *Eur J Oral Sci*. 2016;124(5):459–464. doi:10.1111/eos.12295
16.	**Vervoorn-Vis GM**, Wetselaar P, Koutris M, Visscher CM, Evälahti M, Ahlberg J, Lobbezoo F. Assessment of the progression of tooth wear on dental casts. *J Oral Rehabil*. 2015;42(8):600–604. doi:10.1111/joor.12292
17.	Nikolopoulou M, **Byraki A**, Ahlberg J, Heymans MW, Hamburger HL, De Lange J, Lobbezoo F, Aarab G. Oral appliance therapy versus nasal continuous positive airway pressure in obstructive sleep apnoea syndrome: A randomised, placebo-controlled trial on self-reported symptoms of common sleep disorders and sleep-related problems. *J Oral Rehabil*. 2017;44(6):452–460. doi:10.1111/joor.12505
18.	**Chin Jen Sem JP**, Van der Leeden M, Visscher CM, Britsemmer K, Turk SA, Dekker J, Van Schaardenburg D, Lobbezoo F. Prevalence, course, and associated factors of pain in the temporomandibular joint in early rheumatoid arthritis: Results of a longitudinal cohort study. *J Oral Facial Pain Headache*. 2017;31(3):233–239. doi:10.11607/ofph.1606

No.	Publication
19.	**Thymi M**, Visscher CM, Yoshida-Kohno E, Crielaard W, Wismeijer D, Lobbezoo F. Associations between sleep bruxism and (peri-) implant complications: A prospective cohort study. *BDJ Open*. 2017;3:17003. doi:10.1038/bdjopen.2017.3
20.	**Reyes-Sevilla M**, Kuijs RH, Werner A, Kleverlaan CJ, Lobbezoo F. Comparison of wear between occlusal splint materials and resin composite materials. *J Oral Rehabil*. 2018;45(7):539–544. doi:10.1111/joor.12636
21.	**Kapagiannidou D**, Koutris M, Wetselaar P, Visscher CM, Van der Zaag J, Lobbezoo F. Association between polysomnographic parameters of sleep bruxism and attrition-type tooth wear. *J Oral Rehabil*. 2021;48(6):687–691. doi:10.1111/joor.13149
22.	Delwel S, Maier AB, **Parvaneh D**, Meijers J, Scherder EJ, Lobbezoo F. Chewing efficiency, global cognitive functioning, and dentition: A cross-sectional observational study in older people with mild cognitive impairment or mild to moderate dementia. *Front Aging Neurosci*. 2020;12:225. doi:10.3389/fnagi.2020.00225
23.	Wetselaar P, Lobbezoo F, **De Jong P**, Choudry U, Van Rooijen J, Langerak R. A methodology for evaluating tooth wear monitoring using timed automata modelling. *J Oral Rehabil*. 2020;47(3):353–360. doi:10.1111/joor.12908
24.	**Bousché G**, Koutris M, Su N, Verhoeff MC, Lobbezoo F. Predictors of patients' satisfaction after temporomandibular disorder treatment in a referral clinic. *J Oral Rehabil*. 2024;51(2):266–277. doi:10.1111/joor.13600
25.	**Van de Rijt LJ**, Weijenberg RA, Feast AR, Vickerstaff V, Lobbezoo F, Sampson EL. Oral health and orofacial pain in people with dementia admitted to acute hospital wards: Observational cohort study. *BMC Geriatr*. 2018;18(1):121. doi:10.1186/s12877-018-0810-7
26.	**Verhoeff MC**, Lobbezoo F, Wetselaar P, Aarab G, Koutris M. Parkinson's disease, temporomandibular disorders and bruxism: A pilot study. *J Oral Rehabil*. 2018;45(11):854–863. doi:10.1111/joor.12697
27.	**Sladeckova N**, Verhoeff MC, Koutris M, Lobbezoo F. Bruxism management during tooth wear rehabilitation: A perspective on the temporary use of contingent electrical stimulation. *Dent Med Probl*. 2025;62(4):561–564. doi:10.17219/dmp/207388
28.	**Soffner M**, Koutris M, Baggen J, De Lange J, Lobbezoo F. Diagnosing neuropathic orofacial pain in the general dental practice [in Dutch]. *Ned Tijdschr Tandheelkd*. 2024;131(6):263–269. doi:10.5177/ntvt.2024.06.23101
29.	Baggen JH, **Koevoets AC**, Koutris M, Steegers MA, Lobbezoo F. Chronic temporomandibular disorder pain patients with a history of neuropathic pain treatment: A narrative research on their diagnosis and treatment history. *BMC Oral Health*. 2024;24(1):22. doi:10.1186/s12903-023-03796-0
30.	**Chung J**, Knibbe W, Chattrattrai T, De Jongh A, Lobbezoo F. Network analysis of temporomandibular disorder pain and subject-based bruxism in post-traumatic stress disorder patients. *J Oral Rehabil*. 2025;52(9):1399–1406. doi:10.1111/joor.14007
31.	**Raoof M**, Verhoeff MC, Kooshki R, Aarab G, Lobbezoo F. Self-reported oral moistening disorders in obstructive sleep apnoea: A scoping review. *J Oral Rehabil*. 2024;51(1):226–239. doi:10.1111/joor.13532

* Some postgraduate students were exempted from the research projects based on previous experience. In addition, some manuscripts are still under peer review.

Table 2. Overview of the external examiners invited between 2006 and 2025*

Year	Examiner	Institution	City	Country
2006	Prof. B. Wenneberg	University of Gothenburg	Gothenburg	Sweden
2007	Prof. A. De Laat	Katholieke Universiteit (KU) Leuven	Leuven	Belgium
2008	Prof. S. Palla	University of Zurich	Zurich	Switzerland
2009	Prof. J.C. Türp	University of Basel	Basel	Switzerland
2010	Prof. M. Könönen & Prof. J. Ahlberg	University of Helsinki	Helsinki	Finland
2011	Dr. M.H. Steenks	Utrecht University	Utrecht	Netherlands
2012	Prof. A. Michelotti	University of Naples Federico II	Naples	Italy
2013	Prof. T. List & Prof. P. Alstergren	Malmö University	Malmö	Sweden
2014	Prof. L. Baad-Hansen	Aarhus University	Aarhus	Denmark
2015	Prof. D. Manfredini	University of Siena	Siena	Italy
2016	Prof. A.A. Johansson	University of Bergen	Bergen	Norway
2017	Prof. J. Durham	Newcastle University	Newcastle upon Tyne	United Kingdom
2019	Prof. B. Häggman-Henrikson	Malmö University	Malmö	Sweden
2020	Prof. D. Ettlin	University of Zurich	Zurich	Switzerland
2021	Prof. I. Peroz	Charité – Berlin Medical University	Berlin	Germany
2022	Prof. M.O. Ahlers	CMD-Center Hamburg-Eppendorf	Hamburg	Germany
2023	Prof. A. Wänman	Umeå University	Umeå	Sweden
2024	Prof. P. Svensson	Aarhus University	Aarhus	Denmark
2025	Prof. N. Christidis	Karolinska Institutet	Huddinge	Sweden

* There was no examination in 2018.

Finally, at the end of the program, the students are examined by an external examiner and an (independent) internal examiner. During a full examination day, the students present selected patient cases to the examiners to show their competence in clinical decision-making in interdisciplinary work. In addition, the students present their portfolio, which includes not only their research report/international publication, but also the output of all the teaching components. Based on their performance, students can fail, pass, or pass with distinction, based on rubrics that guide the examiners with their grading. The international examiners are invited based on their expertise, international reputation and profile. They are always affiliated with reputable academic dental institutions from all over Europe. Table 2 provides an overview of the external examiners who were invited between 2006 and 2025.

Conclusion

The international English-language postgraduate Oral Health Sciences (OHS) program, profile Orofacial Pain and Dysfunction (OPD), offers full-time, 3-year training for dentists specializing in an evolving dental/medical discipline that includes conditions like orofacial pain, TMDs, oral movement disorders, tooth wear, and dental sleep disorders. Since its start in 2003, 40 OPD specialists have graduated from the program. Most of them have continued (and continue) to work in the field of OPD and contribute, in various capacities, to the further growth and development of this highly interdisciplinary discipline. We look back on 2 decades of a successful program that continues to evolve toward a bright future.

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References

1. Heir GM. Orofacial pain, the 12th specialty: The necessity. *J Am Dent Assoc*. 2020;151(7):469–471. doi:10.1016/j.adaj.2020.05.002
2. Lobbezoo F, Van der Zaag J, Visscher CM, Naeije M. Oral kinesiology. A new postgraduate programme in the Netherlands. *J Oral Rehabil*. 2004;31(3):192–198. doi:10.1046/j.0305-182X.2003.01244.x
3. Academic Centre for Dentistry Amsterdam (ACTA). Postgraduate Orofacial Pain and Dysfunction Program. https://acta.nl/en/education/more-about/orofacial-pain-and-dysfunction. Accessed December 2, 2025.
4. Peck CC, Goulet JP, Lobbezoo F, et al. Expanding the taxonomy of the diagnostic criteria for temporomandibular disorders. *J Oral Rehabil*. 2014;41(1):2–23. doi:10.1111/joor.12132
5. Schiffman E, Ohrbach R, Truelove E, et al.; International RDC/TMD Consortium Network, International Association for Dental Research; Orofacial Pain Special Interest Group, International Association for the Study of Pain. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for clinical and research applications: Recommendations of the International RDC/TMD Consortium Network and Orofacial Pain Special Interest Group. *J Oral Facial Pain Headache*. 2014;28(1):6–27. doi:10.11607/jop.1151
6. Durham J, Al-Baghdadi M, Baad-Hansen L, et al. Self-management programmes in temporomandibular disorders: Results from an international Delphi process. *J Oral Rehabil*. 2016;43(12):929–936. doi:10.1111/joor.12448
7. Lobbezoo F, Aarab G, Knibbe W, et al. Painful temporomandibular dysfunctions: Diagnosis and treatment [in Dutch]. *Ned Tijdschr Tandheelkd*. 2016;123(11):528–532. doi:10.5177/ntvt.2016.11.15259
8. Durham J, Ohrbach R, Baad-Hansen L, et al.; INfORM. Constructing the brief diagnostic criteria for temporomandibular disorders (bDC/TMD) for field testing. *J Oral Rehabil*. 2024;51(5):785–794. doi:10.1111/joor.13652
9. Soffner M, Koutris M, Baggen J, De Lange J, Lobbezoo F. Diagnosing neuropathic orofacial pain in the general dental practice [in Dutch]. *Ned Tijdschr Tandheelkd*. 2024;131(6):263–269. doi:10.5177/ntvt.2024.06.23101
10. Manfredini D, Häggman-Henrikson B, Al Jaghsi A, et al.; International Network for Orofacial Pain and Related Disorders Methodology. Temporomandibular disorders: INfORM/IADR key points for good clinical practice based on standard of care. *Cranio*. 2025;43(1):1–5. doi:10.1080/08869634.2024.2405298
11. Lobbezoo F, Jacobs R, De Laat A, Aarab G, Wetselaar P, Manfredini D. Chewing on bruxism. Diagnosis, imaging, epidemiology and aetiology [in Dutch]. *Ned Tijdschr Tandheelkd*. 2017;124(6):309–316. doi:10.5177/ntvt.2017.06.16194
12. Lobbezoo F, Jacobs R, De Laat A, Aarab G, Wetselaar P, Manfredini D. Chewing on bruxism: Associations, consequences and management [in Dutch]. *Ned Tijdschr Tandheelkd*. 2017;124(7–8):369–376. doi:10.5177/ntvt.2017.07/08.16195
13. Lobbezoo F, Ahlberg J, Verhoeff MC, et al. The bruxism screener (BruxScreen): Development, pilot testing and face validity. *J Oral Rehabil*. 2024;51(1):59–66. doi:10.1111/joor.13442
14. Manfredini D, Ahlberg J, Aarab G, et al. Standardised tool for the assessment of bruxism. *J Oral Rehabil*. 2024;51(1):29–58. doi:10.1111/joor.13411
15. Verhoeff MC, Lobbezoo F, Ahlberg J, et al. Updating the bruxism definitions: Report of an international consensus meeting. *J Oral Rehabil*. 2025;52(9):1335–1342. doi:10.1111/joor.13985
16. Wetselaar P, Lobbezoo F. The tooth wear evaluation system: A modular clinical guideline for the diagnosis and management planning of worn dentitions. *J Oral Rehabil*. 2016;43(1):69–80. doi:10.1111/joor.12340
17. Wetselaar P, Wetselaar-Glas MJ, Katzer LD, Ahlers MO. Diagnosing tooth wear, a new taxonomy based on the revised version of the Tooth Wear Evaluation System (TWES 2.0). *J Oral Rehabil*. 2020;47(6):703–712. doi:10.1111/joor.12972
18. Wetselaar P, Lobbezoo F, De Vries R, Mehta SB, Opdam NJ, Loomans BA. Developing diagnostic criteria for tooth wear, a preliminary beta version based on expert opinion, and a narrative literature review. *J Oral Rehabil*. 2023;50(10):1030–1042. doi:10.1111/joor.13499
19. Lobbezoo F, Aarab G, Wetselaar P, Hoekema A, De Lange J, De Vries N. A new definition of dental sleep medicine. *J Oral Rehabil*. 2016;43(10):786–790. doi:10.1111/joor.12421
20. Nederlandse Vereniging van Artsen voor Longziekten en Tuberculose. Richtlijn Diagnostiek en Behandeling van Obstructief slaapapneu (OSA) bij volwassenen, 2017. https://www.nvalt.nl/kwaliteit/richtlijnen/slaap. Accessed December 2, 2025.

Original paper

Trends in unmet denture needs among Polish seniors with partial or complete edentulism: A comparative analysis of the PolSenior (2009) and PolSenior2 (2019) surveys

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D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. Tooth loss significantly affects health and quality of life of older adults. Removable dentures are the most common solution for restoring oral function and aesthetics. In Poland, a significant portion of elderly population suffers from edentulism or partial tooth loss. However, not all of these individuals receive the prosthetic treatment they need. The measurement of unmet denture needs, defined as the proportion of individuals with tooth loss who lack appropriate dentures, offers critical insight into disparities in the access to oral healthcare.

Objectives. The aim of the study was to compare changes in unmet denture needs among Polish seniors over a decade and to assess patterns in denture use among those who received prosthetic treatment, taking into account sociodemographic factors such as sex, age, education, place of residence, and financial status.

Material and methods. The present study analyzed data from 2 nationally representative cross-sectional surveys, PolSenior (2009) and PolSenior2 (2019), comprising 4,712 and 4,075 participants aged ≥65 years, respectively. The individuals were categorized based on their dental status, denture use and sociodemographic characteristics. Unmet denture need was defined as the absence of complete or partial dentures among individuals with edentulism or partial tooth loss. The χ^2 test and age standardization were implemented for the purpose of the statistical analysis. The level of significance was set at $p < 0.05$.

Results. The proportion of edentulous and partially dentate individuals without dentures decreased from 19.4% in 2009 to 12.3% in 2019. The most pronounced improvements were noted among women, urban residents and individuals aged 85 years and older. Despite these gains, disparities by sex, place of residence, education level, and financial status persisted. Among individuals who owned dentures, denture use remained consistently high in both survey waves, exceeding 94%, with no significant differences between the study periods.

Conclusions. The unmet need for dentures among Polish seniors has decreased over time, indicating an improvement in the accessibility to prosthetic care. However, sociodemographic disparities remain, highlighting the necessity for targeted public health strategies aimed at mitigating barriers to dental rehabilitation. The high rates of denture use confirm the enduring functional and psychosocial value of prosthetic treatment in older populations.

Keywords: dental health, dentures, demographic trends, geriatric dentistry, older adult population

Highlights

- Unmet denture needs among Polish seniors decreased from 19.4% in 2009 to 12.3% in 2019.
- The greatest improvements were observed among women, urban residents and individuals aged 85 years and older.
- Denture use among individuals who owned dentures remained consistently high, exceeding 94% in both survey periods.
- Disparities in denture coverage and use persist, particularly among men, rural residents and those with lower education attainment.
- Public health interventions should focus on improving access to prosthetic care in underserved populations.

Introduction

Tooth loss is a major public health concern among older adults, with far-reaching functional, nutritional, psychological, and social consequences. Globally, approx. 20% of the population over the age of 60 suffers from edentulism, often due to years of health neglect and limited access to dental care.[1,2]

The loss of natural dentition significantly impairs masticatory function.[3] Studies show that older adults without teeth often consume softer, processed foods, which can lead to digestive and nutrient absorption issues, increasing the risk of malnutrition and conditions such as cardiovascular disease, osteoporosis and metabolic disorders.[4,5]

Beyond physical health, edentulism also affects psychosocial well-being. The condition leads to changes in facial structure, contributing to premature aging of facial features and lip collapse, as well as negatively impacting self-esteem and increasing the risk of social isolation.[6,7] These challenges are observed not only in low-resource settings but also in developed countries, where high treatment costs and the lack of comprehensive geriatric dental programs contribute to persistently high rates of edentulism.

In Europe, despite improvements in access to dental services, tooth loss remains prevalent among older populations, particularly in Eastern Europe, where edentulism rates exceed those observed in Western countries.[8] In Poland, the 2019 PolSenior2 survey revealed that 36.1% of individuals over the age of 65 are completely edentulous, highlighting the severity of edentulism in Poland despite significant improvements over the past decade.[9]

Conventional dentures represent the most common and inexpensive solution for restoring lost teeth, allowing individuals to improve oral function, enhance phonetics, engage socially, and maintain an aesthetically acceptable appearance.[10] Conventional dentures in this study refer exclusively to removable complete and partial dentures, not implant-supported or fixed prostheses. Dentures improve chewing ability, which is important for digestion and absorption of nutrients.[11] Elderly individuals who wear dentures generally exhibit better nutritional status, as evidenced by higher body mass index (BMI) measurements.[12] Notably, the Polish national healthcare system provides complete and partial dentures (for cases involving more than 5 missing teeth in a single arch) at no cost every 5 years, which likely contributes to the observed improvements in denture coverage and utilization rates.

The advantages of dentures extend beyond physical functionality, encompassing psychological and social dimensions. Studies have demonstrated that denture wearers participate more actively in social life, experience diminished feelings of loneliness and are at a reduced risk of depression.[13–16]

The present cross-sectional study compares data from the PolSenior (2009) and PolSenior2 (2019) surveys to assess changes in the proportion of older adults (≥65 years of age) with unmet denture needs, as well as trends in denture use among those who received prosthetic treatment.

PolSenior was the first large-scale, multidisciplinary project in Poland dedicated to the study of aging, covering medical, psychological, functional, economic, and social aspects of the lives of older adults. A decade later, PolSenior2 continued this work, using a consistent methodology and introducing additional modules, including a focus on oral health. These studies offer a unique opportunity to assess trends in various domains of aging over time. The third edition of the project, PolSenior3, is currently underway and aims to further evaluate the evolving needs of the older population in Poland.

Material and methods

The study draws on data from 2 nationwide cross-sectional surveys: PolSenior (2009); and PolSenior2 (2019). Both projects aimed to assess the health status and socioeconomic conditions of Poland's aging population. A particular emphasis was placed on oral health, including denture use, enabling a decade-long comparison of trends and disparities. The authors were not involved in the original data collection, but they obtained formal permission to use the datasets for comparative analysis.

Participants were divided into age cohorts, enabling precise comparisons across age groups. A balanced sex ratio was maintained in both studies, facilitating reliable comparisons between male and female subjects.

A three-stage, stratified, proportional random sampling method was implemented in both projects to ensure national representativeness. In the first stage, administrative units (rural, urban and mixed municipalities) were selected; in the second stage, specific streets or villages within the selected municipalities were chosen; and in the third stage, individual participants were randomly selected based on the national PESEL (Personal Identification Number) registry, which is managed by the Ministry of the Interior and Administration of Poland. To enable detailed subgroup analyses, oversampling of the oldest age groups was implemented.[17,18]

The participants were classified based on their dental status as edentulous (no teeth), partially dentate (1–19 teeth), or functionally dentate (20 teeth or more). For the purpose of this study, functionally dentate individuals were excluded, as they typically do not require removable prosthetic treatment. The classification of dental status and prosthesis use was based exclusively on structured, self-reported questionnaire responses obtained during face-to-face interviews with trained fieldworkers. No clinical dental examinations were performed to verify the number of remaining natural teeth or the presence of dentures. The analysis focused on assessing both the unmet need for dentures and the actual use of complete and partial dentures among edentulous and partially dentate individuals.

The sociodemographic variables examined in relation to prosthetic needs and denture use included the following:

- age group (65–74; 75–84; ≥85 years);
- sex (female; male);
- education level (primary; vocational; secondary; higher);
- place of residence (rural; town with <50,000 residents; city with 50,000–200,000 residents; city with >200,000 residents);
- self-reported financial status (able to afford everything; can afford with saving; difficulties paying for food and clothing).

The final analytical sample comprised 4,712 participants aged 65+ from the PolSenior survey and 4,075 individuals from PolSenior2. The groups were harmonized to ensure comparability across the 2 time points.

Statistical analysis

The data collected in both studies was subjected to comparative analysis using descriptive statistics. The χ^2 tests were applied to assess differences in unmet need for dentures between 2009 and 2019 using categorical variables, and age standardization was performed to account for demographic changes over the analyzed period. Statistical analyses were conducted using R software (v. 3.6.3; https://www.r-project.org) and SAS (v. 9.4; SAS, Cary, USA). The results were presented as percentages with 95% confidence intervals (*CI*). Sampling weights, which reflect the complex sampling design and post-stratification adjustments, were applied to align the sample distribution by age and sex with the Polish population in 2019. The significance level was set at $p < 0.05$.

Ethical considerations

Both studies were conducted in accordance with established ethical guidelines, and all participants provided written informed consent. The projects were approved by the Independent Bioethics Committee of the Medical University of Gdańsk, Poland (approval No. NKBBN/257/2017), ensuring compliance with local regulations and institutional requirements.[17,18]

Results

Overall comparison of unmet denture needs in older adults (≥65 years)

A comparison of results from the PolSenior (2009) and PolSenior2 (2019) surveys revealed a statistically significant decrease in the prevalence of unmet denture needs among older adults in Poland. The proportion of edentulous and partially dentate individuals who do not use dentures decreased from 19.4% (95% *CI*: 16.4–22.5%) in 2009 to 12.3% (95% *CI*: 10.7–13.9%) in 2019, reflecting improved prosthetic service coverage (Table 1).

Unmet denture needs by sex

In both studies, female subjects consistently exhibited a lower level of unmet denture needs compared to male individuals. The proportion of women with unmet need for dentures decreased from 16.3% (95% *CI*: 12.5–20.0%) in 2009 to 8.1% (95% *CI*: 6.3–9.8%) in 2019. Among men, the unmet need declined from 24.4% (95% *CI*: 20.9–27.9%) to 19.2% (95% *CI*: 16.5–21.9%) over the same period (Table 1). Although improvement was observed in both sexes, the disparity between men and women remained.

Unmet denture needs by age group

A subsequent age-stratified analysis demonstrated a marked reduction in unmet denture needs, especially among older women. In the 65–74 age group of female subjects, the unmet need decreased from 14.3% (95% *CI*: 9.4–19.1%) to 6.4% (95% *CI*: 4.1–8.7%). In the 85+ group of women, the unmet need exhibited a decline from 25.3% (95% *CI*: 17.3–33.4%) to 10.2% (95% *CI*: 7.3–13.2%). Among men, a decrease was noted in each age group; however, these changes did not reach statistical significance (Table 1).

Table 1. Prevalence of overall unmet denture needs among Polish individuals aged ≥65 years in the PolSenior and PolSenior2 studies

Variable			PolSenior	PolSenior2
Overall unmet denture needs			19.4 (16.4–22.5)*	12.3 (10.7–13.9)*
Sex		female	16.3 (12.5–20.0)*	8.1 (6.3–9.8)*
		male	24.4 (20.9–27.9)	19.2 (16.5–21.9)
Age	65–74	female	14.3 (9.4–19.1)*	6.4 (4.1–8.7)*
		male	24.4 (19.3–29.5)	20.0 (16.0–24.0)
	75–84	female	15.6 (10.9–20.2)	9.6 (6.3–12.8)
		male	23.1 (18.1–28.2)	17.9 (13.8–22.1)
	>85	female	25.3 (17.3–33.4)*	10.2 (7.3–13.2)*
		male	28.5 (19.7–37.3)	18.3 (13.8–22.8)
Place of residence	rural areas	female	22.7 (15.0–30.3)*	11.3 (8.1–14.5)*
		male	35.9 (30.5–41.2)*	24.6 (20.2–29.0)*
	town with <50,000 residents	female	12.4 (8.8–16.0)*	5.3 (3.2–7.5)*
		male	24.5 (19.0–30.1)	21.6 (16.4–26.7)
	city with 50,000–200,000 residents	female	14.4 (10.0–18.8)	6.9 (3.5–10.3)
		male	16.8 (10.3–23.2)	11.5 (7.2–15.9)
	city with >200,000 residents	female	7.9 (5.0–10.8)	4.9 (1.5–8.3)
		male	14.5 (8.5–20.6)	11.9 (7.6–16.1)
Education level	primary	female	19.4 (14.1–24.7)	12.5 (9.6–15.4)
		male	37.0 (32.2–41.7)	33.7 (27.9–39.5)
	vocational	female	15.6 (5.0–26.1)	5.0 (2.1–8.0)
		male	20.4 (12.8–28.0)	15.2 (11.0–19.4)
	secondary	female	8.3 (5.1–11.5)	4.6 (2.7–6.4)
		male	12.7 (8.5–16.9)	13.2 (9.8–16.6)
	higher education	female	12.1 (4.7–19.4)	7.0 (1.7–12.3)
		male	12.0 (7.0–17.1)	5.8 (2.6–9.1)

Data presented as percentages (%), with the 95% confidence intervals (*CIs*) shown in brackets. Statistically significant differences between the PolSenior and PolSenior2 studies are marked with an asterisk ($p < 0.05$).

Unmet denture needs by place of residence

Across both time points, urban residents exhibited a consistently lower prevalence of unmet denture needs compared to their rural counterparts. In rural areas, the proportion of women without dentures decreased from 22.7% (95% *CI*: 15.0–30.3%) in 2009 to 11.3% (95% *CI*: 8.1–14.5%) in 2019, while the proportion of male subjects with unmet denture needs declined from 35.9% (95% *CI*: 30.5–41.2%) to 24.6% (95% *CI*: 20.2–29.0%). In small urban centers (<50,000 residents), the unmet need among women decreased from 12.4% (95% *CI*: 8.8–16.0%) to 5.3% (95% *CI*: 3.2–7.5%) (Table 1).

Unmet denture needs by education level

Lower levels of education were associated with higher unmet denture needs in both survey waves. Among individuals with a primary education, the unmet need remained relatively high, while the lowest levels were observed among those with a higher education. Despite an observed downward trend across all education levels between 2009 and 2019, the differences did not reach statistical significance (Table 1). The association was particularly notable among men, where educational disparities were more pronounced.

Unmet denture needs by financial situation

A significant improvement in denture access was observed among respondents who reported being able to make purchases when saving. In this group, the unmet need for dentures decreased from 17.5% (95% *CI*: 13.7–21.3%) in 2009 to 11.6% (95% *CI*: 9.5–13.7%) in 2019. Financial disparities were evident in the PolSenior2 survey. Individuals who reported they can afford everything had an unmet need of 8.8% (95% *CI*: 6.1–11.5%), while those with difficulties paying for food and clothing had a significantly higher unmet need of 15.2% (95% *CI*: 12.1–18.2%) (Table 2).

Unmet needs for partial dentures

Among individuals with partial tooth loss (1–19 teeth), the prevalence of unmet need for partial dentures decreased significantly from 25.0% (95% *CI*: 19.6–30.3%) in 2009 to 16.0% (95% *CI*: 13.6–18.5%) in 2019 (Table 3).

Table 2. Prevalence of unmet denture needs and denture utilization among denture wearers in the PolSenior and PolSenior2 studies, stratified by financial status

Variable		Financial status	PolSenior	PolSenior2
Unmet denture needs	overall	can afford everything	14.1 (10.2–18.0)*	8.8 (6.1–11.5)*
		can afford when saving	17.5 (13.7–21.3)	11.6 (9.5–13.7)
		difficulties paying for food and clothing	21.6 (16.7–26.5)*	15.2 (12.1–18.2)*
	edentulous individuals	can afford everything	6.5 (2.9–10.0)	5.5 (0.4–10.5)
		can afford when saving	8.4 (5.8–11.1)	5.7 (3.5–7.8)
		difficulties paying for food and clothing	11.1 (7.4–14.8)	9.4 (5.8–13.0)
	partially dentate individuals	can afford everything	20.1 (13.7–26.4)*	10.7 (6.8–14.6)*
		can afford when saving	23.7 (16.8–30.5)	15.5 (12.2–18.7)
		difficulties paying for food and clothing	27.8 (20.9–34.8)*	20.8 (15.9–25.7)*
Denture use	overall	can afford everything	96.3 (94.0–98.6)	96.1 (93.9–98.3)
		can afford when saving	94.9 (93.1–96.8)	95.8 (94.6–97.0)
		difficulties paying for food and clothing	93.9 (92.0–95.7)	92.9 (90.9–94.9)
	edentulous individuals	can afford everything	98.8 (97.6–100)	96.6 (94.1–99.1)
		can afford when saving	95.0 (92.1–97.9)	97.2 (96.0–98.5)
		difficulties paying for food and clothing	93.6 (91.0–96.1)	94.5 (92.0–97.0)
	partially dentate individuals	can afford everything	93.5 (89.0–98.0)	95.8 (92.6–99.0)
		can afford when saving	95.0 (92.6–97.3)	94.7 (92.9–96.6)
		difficulties paying for food and clothing	93.9 (90.9–96.9)	91.0 (87.8–94.2)

Data presented as % (95% *CI*).

Table 3. Prevalence of unmet need for partial and complete dentures among Polish individuals aged ≥65 years in the PolSenior and PolSenior2 studies

Variable			Partial dentures		Complete dentures	
			PolSenior	PolSenior2	PolSenior	PolSenior2
Overall unmet denture needs			25.0 (19.6–30.3)*	16.0 (13.6–18.5)*	11.4 (9.0–13.8)*	7.2 (5.4–9.0)*
Sex		female	20.6 (14.3–26.8)*	10.5 (7.9–13.1)*	10.4 (7.7–13.2)*	5.2 (3.0–7.5)*
		male	30.8 (25.0–36.7)	23.7 (19.9–27.5)	13.3 (9.7–16.8)	11.3 (8.2–14.4)
Age	65–74	female	17.4 (10.3–24.6)	8.1 (4.8–11.4)	8.1 (3.3–12.8)	3.3 (0.6–6.1)
		male	30.1 (21.7–38.5)	23.4 (18.3–28.5)	11.7 (6.9–16.4)	11.1 (5.2–17.0)
	75–84	female	21.7 (13.5–29.9)	13.3 (8.9–17.7)	9.3 (5.4–13.3)	6.0 (1.4–10.6)
		male	29.6 (22.7–36.5)	24.1 (17.8–30.5)	14.9 (9.3–20.4)	10.7 (6.9–14.6)
	>85	female	38.2 (22.2–54.2)	17.8 (12.0–23.7)	18.1 (12.3–23.9)*	7.1 (3.7–10.4)*
		male	42.3 (25.6–58.9)	25.2 (18.2–32.2)	17.3 (12.5–22.2)	13.2 (7.3–19.1)
Place of residence	rural areas	female	32.0 (18.9–45.2)	15.1 (10.5–19.8)	13.6 (9.1–18.1)	7.9 (3.8–12.1)
		male	46.1 (36.0–56.2)	30.5 (24.2–36.8)	22.2 (15.5–28.9)	13.9 (9.4–18.4)
	town with <50,000 residents	female	15.0 (9.2–20.8)	7.3 (3.7–11.0)	8.3 (3.6–13.0)	2.7 (0.0–5.6)
		male	31.0 (22.9–39.0)	25.9 (18.7–33.2)	13.5 (7.7–19.2)	14.5 (5.5–23.4)
	city with 50,000–200,000 residents	female	17.5 (8.5–26.5)	10.7 (5.6–15.7)	9.4 (2.3–16.6)	1.8 (0.0–3.7)
		male	18.7 (12.7–24.6)	17.1 (11.1–23.1)	7.5 (1.5–13.6)	3.7 (0.2–7.2)
	city with >200,000 residents	female	9.6 (5.7–13.6)	5.9 (0.5–11.4)	4.4 (1.8–7.0)	3.0 (1.6–4.5)
		male	18.9 (10.2–27.6)	12.4 (7.1–17.6)	4.7 (0.8–8.5)	10.7 (5.8–15.6)
Education level	primary	female	28.3 (18.5–38.0)	19.5 (14.0–25.1)	11.9 (8.7–15.1)	7.5 (4.6–10.3)
		male	51.2 (43.8–58.6)	46.9 (38.1–55.6)	19.0 (13.2–24.9)	17.3 (11.4–23.2)
	vocational	female	20.5 (6.5–34.4)	5.3 (1.7–8.9)	9.3 (0.6–18.0)	4.7 (0.0–9.9)
		male	28.5 (19.6–37.5)	17.4 (12.3–22.5)	9.4 (2.5–16.3)	10.9 (4.4–17.3)
	secondary	female	10.4 (6.1–14.6)	6.5 (3.5–9.5)	2.0 (0.1–3.8)	1.3 (0.3–2.4)
		male	12.0 (6.4–17.7)	16.8 (12.3–21.3)	6.9 (2.2–11.5)	6.0 (1.2–10.8)
	higher education	female	13.0 (3.3–22.7)	9.7 (2.7–16.7)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
		male	14.7 (7.9–21.6)	6.6 (2.3–10.8)	3.3 (0.0–7.5)	3.9 (0.0–8.3)

Data presented as % (95% *CI*). Statistically significant differences between the PolSenior and PolSenior2 studies are marked with an asterisk ($p < 0.05$).

The most substantial improvement was observed among women, where the unmet needs declined from 20.6% (95% *CI*: 14.3–26.8%) to 10.5% (95% *CI*: 7.9–13.1%).

In PolSenior2, financial status was significantly associated with unmet denture needs. Among those who reported they can afford everything, the unmet need for partial dentures was 10.7% (95% *CI*: 6.8–14.6%), compared to 20.8% (95% *CI*: 15.9–25.7%) among individuals who experienced difficulties paying for food and clothing.

Unmet needs for complete dentures

In the edentulous group (0 teeth), the proportion of individuals without complete dentures decreased significantly from 11.4% (95% *CI*: 9.0–13.8%) in 2009 to 7.2% (95% *CI*: 5.4–9.0%) in 2019 (Table 3).

Among women, the unmet needs declined from 10.4% (95% *CI*: 7.7–13.2%) to 5.2% (95% *CI*: 3.0–7.5%), with the most pronounced improvement observed in the 85+ age group, where the rate fell from 18.1% (95% *CI*: 12.3–23.9%) in 2009 to 7.1% (95% *CI*: 3.7–10.4%) in 2019.

Denture use among denture wearers

Denture use among Polish older adults, as presented in Table 4, remained consistently high over the analyzed decade, with no statistically significant differences observed between the PolSenior (2009) and PolSenior2 (2019) surveys. The overall utilization rates were 94.5% (95% *CI*: 93.3–95.7%) in 2009 and 94.9% (95% *CI*: 93.9–95.8%) in 2019, highlighting the widespread use of dentures across diverse demographic groups and socioeconomic conditions. Both surveys revealed very high usage rates for complete and partial dentures, with complete dentures being used slightly more frequently than partial ones in most subgroups.

Sex differences

Overall denture use was consistently higher among women than men. In 2019, 95.8% (95% *CI*: 94.9–96.7%) of women reported regular denture use, compared to 93.1% (95% *CI*: 91.0–95.2%) of men. This discrepancy

Table 4. Prevalence of overall, complete and partial denture utilization among Polish individuals aged ≥65 years in the PolSenior and PolSenior2 studies

Variable			Overall denture use		Complete denture use		Partial denture use	
			PolSenior	PolSenior2	PolSenior	PolSenior2	PolSenior	PolSenior2
Overall unmet denture needs			94.5 (93.3–95.7)	94.9 (93.9–95.8)	94.6 (92.8–96.3)	96.1 (95.0–97.2)	94.4 (92.9–95.9)	93.9 (92.4–95.3)
Sex		female	95.0 (93.5–96.5)	95.8 (94.9–96.7)	94.2 (92.0–96.5)	96.8 (95.6–97.9)	95.9 (94.3–97.5)	94.9 (93.5–96.4)
		male	93.7 (91.9–95.5)	93.1 (91.0–95.2)	95.2 (93.1–97.4)	94.6 (92.0–97.2)	92.0 (89.0–95.0)	92.1 (89.2–95.1)
Age	65–74	female	97.3 (95.8–98.8)	97.4 (96.2–98.6)	97.9 (96.2–99.5)	99.5 (98.7–100.0)	96.8 (94.9–98.7)	96.2 (94.3–98.1)
		male	93.8 (91.2–96.4)	93.1 (90.2–96.1)	95.7 (92.5–99.0)	95.6 (91.2–100.0)	92.0 (87.8–96.3)	92.0 (87.9–96.1)
	75–84	female	92.5 (88.8–96.3)	95.5 (93.8–97.3)	91.2 (85.4–97.1)	95.8 (93.4–98.2)	94.8 (91.4–98.2)	95.2 (92.5–98.0)
		male	94.2 (92.1–96.3)	93.8 (91.8–95.9)	95.0 (92.5–97.6)	94.0 (90.8–97.2)	93.1 (89.5–96.7)	93.6 (90.6–96.7)
	>85	female	90.9 (87.9–94.0)	91.0 (87.8–94.1)	90.5 (86.4–94.6)	93.8 (90.9–96.6)	91.9 (86.9–96.9)	83.4 (75.6–91.2)
		male	90.8 (87.8–93.8)	90.9 (86.6–95.2)	92.7 (89.6–95.8)	93.0 (88.2–97.8)	87.1 (81.9–92.3)	87.5 (80.7–94.4)
Place of residence	rural areas	female	92.6 (90.3–95.0)	93.5 (91.5–95.5)	92.0 (88.5–95.4)	95.0 (92.8–97.1)	93.6 (91.0–96.3)	91.1 (87.3–94.9)
		male	91.5 (88.3–94.8)	91.8 (88.0–95.6)	92.5 (88.0–97.0)	90.0 (83.5–96.4)	89.8 (85.3–94.4)	94.1 (91.1–97.1)
	town with <50,000 residents	female	98.9 (97.6–100.0)	96.9 (94.9–99.0)	98.7 (96.6–100.0)	98.3 (96.8–99.9)	99.3 (97.8–100.0)	95.9 (92.6–99.2)
		male	92.5 (87.5–97.6)	90.8 (86.2–95.4)	96.1 (92.3–99.9)	95.9 (92.6–99.1)	88.3 (79.2–97.4)	88.0 (81.3–94.8)
	city with 50,000–200,000 residents	female	97.8 (96.0–99.6)	97.6 (96.5–98.8)	99.0 (97.8–100.0)	99.0 (97.8–100.0)	97.0 (93.9–100.0)	96.8 (95.0–98.5)
		male	95.2 (91.8–98.6)	95.3 (92.1–98.5)	98.2 (96.1–100.0)	97.1 (94.2–100.0)	93.0 (87.1–98.8)	94.3 (89.7–98.9)
	city with >200,000 residents	female	98.6 (95.7–100.0)	96.4 (93.0–99.8)	100 (100–100.0)	97.2 (93.0–100.0)	98.1 (94.3–100.0)	96.0 (91.5–100.0)
		male	98.2 (96.5–100.0)	96.0 (93.3–98.8)	98.5 (96.9–100.0)	99.4 (98.5–100.0)	98.1 (95.6–100.0)	94.7 (90.9–98.5)
Education level	primary	female	92.8 (89.7–95.9)	94.8 (93.2–96.4)	91.7 (87.0–96.3)	96.1 (94.3–97.9)	94.8 (92.1–97.6)	93.1 (90.3–95.8)
		male	93.4 (91.1–95.6)	92.9 (90.0–95.8)	94.0 (91.0–97.1)	93.1 (88.2–98.0)	92.6 (89.0–96.2)	92.7 (88.3–97.1)
	vocational	female	95.1 (92.0–98.2)	96.0 (93.9–98.0)	94.4 (91.3–97.5)	96.2 (93.1–99.3)	96.1 (92.0–100.0)	95.8 (92.8–98.8)
		male	95.0 (92.4–97.6)	94.5 (90.7–98.2)	94.7 (90.8–98.6)	96.8 (93.0–100.0)	95.0 (91.4–98.5)	92.8 (87.1–98.5)
	secondary	female	95.2 (92.9–97.5)	96.5 (94.0–99.1)	97.1 (95.0–99.2)	97.9 (96.0–99.8)	92.3 (87.5–97.1)	95.5 (91.3–99.6)
		male	90.6 (84.7–96.5)	92.9 (88.6–97.3)	93.4 (86.7–100.0)	95.2 (90.5–99.8)	87.4 (78.8–96.1)	91.1 (85.5–96.7)
	higher education	female	98.8 (98.1–99.4)	97.2 (95.9–98.5)	98.3 (97.0–99.5)	98.6 (97.4–99.9)	99.3 (98.7–99.9)	96.4 (94.4–98.4)
		male	95.2 (91.9–98.6)	92.4 (86.1–98.7)	98.6 (97.1–100)	94.8 (90.2–99.4)	92.4 (85.8–99.0)	91.4 (83.1–99.7)

Data presented as % (95% *CI*).

in outcomes was observed across both complete and partial dentures, and persisted across various demographic groups, including age, education and place of residence (Table 4).

Influence of education

Denture use increased along with education levels. Significant differences were noted between individuals with a primary education and those with a higher education. In 2019, the denture utilization rate among women with a higher education reached 96.4% (95% *CI*: 93.0–99.8%) compared to 93.5% (95% *CI*: 91.5–95.5%) for those with a primary education only. Among the male population, the corresponding values were 96.0% (95% *CI*: 93.3–98.8%) for those with a higher education compared to 91.8% (95% *CI*: 88.0–95.6%) for those with a primary education (Table 4).

Urban–rural differences

Higher denture use was observed in larger urban areas compared to rural regions. In 2009, denture utilization in rural areas was 92.8% (95% *CI*: 89.7–95.9%) among women, compared to 98.8% (95% *CI*: 98.1–99.4%) in cities with populations exceeding 200,000. A similar trend was observed in the male population and in both PolSenior surveys. This pattern was consistent across both complete and partial dentures (Table 4).

Age-related trends

Denture use declined with advancing age. In 2019, the use of dentures among individuals aged 65–74 years reached 97.4% (95% *CI*: 96.2–98.6%) for women and 93.1% (95% *CI*: 90.2–96.1%) for men. In the 85+ group, the prevalence of denture use decreased to 91.0% (95% *CI*: 87.8–94.1%) among women and to 90.9% (95% *CI*: 86.6–95.2%) among men. The most pronounced decline was observed for partial dentures in the oldest age group (85+). In 2019, the use of partial dentures was 83.4% (95% *CI*: 75.6–91.2%) among women and 87.5% (95% *CI*: 80.7–94.4%) among men.

Financial factors

The PolSenior2 survey revealed that there were no significant differences in denture use between individuals with different financial statuses. However, the results of the PolSenior survey noted a statistically significant difference among edentulous individuals. Participants who reported having sufficient financial resources exhibited a denture utilization rate of 98.8% (95% *CI*: 97.6–100.0%), compared to 93.6% (95% *CI*: 91.0–96.1%) observed among those encountering difficulties paying for food and clothing (Table 2).

Complete vs. partial denture usage

Complete dentures demonstrated slightly higher utilization rates compared to partial dentures across all demographic categories. For instance, complete denture use in 2019 was 96.1% (95% *CI*: 95.0–97.2%) compared to 93.9% (95% *CI*: 92.4–95.3%) for partial dentures (Table 4).

Discussion

The observed decrease in unmet denture needs among Polish seniors — from 19.4% in 2009 to 12.3% in 2019 — indicates notable improvements in access to dental prosthetic care over the past decade. This growth is likely the result of multiple factors, including expanded access to affordable dental care, increased awareness of the importance of oral health among older adults, and health policies that integrate oral care into comprehensive eldercare.

The consistently high rates of denture use observed in both surveys (>94%) among denture wearers confirm that prosthetic treatment is widely used and valued once obtained. This outcome is indicative of the efficacy and acceptability of prosthetic appliances, underscoring the importance of maintaining high standards of fabrication, clinical follow-up and patient education.[19]

The observed trend in Poland aligns with global improvements in senior dental health, where greater access to dental services and oral health education has led to better outcomes for aging populations. In many countries, the implementation of dental subsidies and public education initiatives have increased the accessibility to dental prosthetics, improving seniors' quality of life by preserving oral functionality and aesthetics. The Polish data offers further insight into these global trends and underscores the impact of systemic support in promoting oral health equity among the elderly population. Previous comparative analyses using the Survey of Health, Ageing and Retirement in Europe (SHARE) dataset have identified Poland, Germany, Austria, Ireland, Belgium, and the Netherlands as countries with similarly elevated rates of prosthetic rehabilitation among older adults.[20,21]

In contrast, Scandinavian and Southern European countries, such as Sweden, Greece, France, and Italy, report lower rates of denture usage. A study from Norway illustrates substantial regional disparities within a national context. In Oslo and adjacent counties, the majority of older adults retained their natural teeth, while in northern regions, 65.7% of seniors relied exclusively on complete dentures.[22]

Expanding beyond the European context, recent research from Asia and the Middle East reinforces the global variability in denture wearing patterns. Yuan et al. reported that 67.1% of urban edentulous seniors in China wore dentures, compared to only 51.1% in rural areas.[23] This urban–rural disparity was primarily driven by differences

in education, income, chronic disease burden, and health behavior, especially physical activity.[23]

In Jordan, Mukatash Nimri and Jebreen found that rural elderly individuals exhibited significantly higher needs for denture replacement compared to their urban counterparts (69.5% vs. 31.6%, respectively). Moreover, nearly half of the rural elderly (47%) reported dissatisfaction with denture fit or function.[24] These patterns highlight persistent gaps in prosthetic care access and maintenance, particularly in under-resourced regions.[24]

Taken together, these international comparisons underscore that denture wearing among older adults is influenced not only by clinical need (e.g., edentulism), but also by systemic and sociocultural factors. Poland's high denture prevalence is consistent with global patterns observed in countries with similar socioeconomic and healthcare structures, thereby reinforcing the need for integrated prosthetic and preventive care strategies that are context-sensitive and equity-focused.

In the present study, a notable and persistent sex disparity was observed, with women consistently exhibiting lower unmet denture needs than men. This phenomenon may be attributed to sex differences in health-seeking behaviors and perceptions of self-care.[25,26] Additionally, societal expectations regarding appearance and the impact of tooth loss on facial aesthetics may make dentures more appealing for women. Previous studies have highlighted the psychosocial benefits of denture use, particularly in relation to self-image and quality of life.[13,27]

In 2009, the unmet denture needs were highest among the oldest age group (85+), likely due to a combination of the following factors: limited historical access to dental care; lower health literacy; and physical barriers such as mobility issues. However, by 2019, these disparities substantially narrowed. The unmet needs among the oldest individuals sharply declined, especially among women. This shift may partly reflect cohort turnover, as individuals who had already received dentures in middle age transitioned into older age groups while continuing to use them.

The age-related improvement in the use of dentures over recent decades suggests an increase in both awareness and service availability. Nevertheless, the utilization of existing dentures exhibited a slight decline with advancing age, which is likely influenced by reduced social activity, general frailty and physical limitations associated with wearing and maintaining dentures.

A persistent urban–rural gap remains evident. Seniors living in rural areas consistently exhibited higher unmet denture needs compared to those from cities. This disparity may be attributed to the limited number of dental clinics in rural areas, which results in reduced accessibility to dental services. For elderly individuals with mobility challenges, the lack of support from close relatives or limited transportation options to urban centers further exacerbates the difficulty of accessing dental care. Consequently, many individuals forgo acquiring dentures due to significant logistical barriers.[28,29] Moreover, the lifestyle in rural areas differs from that in cities. Social pressure related to appearance is often less pronounced in rural settings, potentially diminishing the motivation of individuals with missing teeth to seek prosthetic solutions.

Despite these barriers, rural regions witnessed substantial progress between 2009 and 2019, marked by an approx. 11% reduction in the prevalence of unmet denture needs among both women and men. However, urban populations continue to demonstrate superior outcomes.

Education level was strongly associated with prosthetic coverage. Seniors with higher education had significantly lower unmet denture needs. This trend reflects greater awareness of oral health and a better understanding of the consequences of tooth loss among individuals with higher education. Education level has also been demonstrated to correlate with financial stability and higher retirement benefits, which facilitate better access to dental care.[30,31] These findings underscore the critical role of education in promoting oral health and suggest that public health initiatives should prioritize oral health education, with a particular emphasis on populations with lower levels of educational attainment.

Financial status has a significant influence on the decision to undergo dental prosthesis treatment. This phenomenon was particularly evident in the 2019 study, which revealed that the replacement of missing teeth was considerably more prevalent among the most financially stable seniors compared to those in the most challenging economic situations. The national healthcare system in Poland provides reimbursement for complete dentures for edentulous individuals, which likely explains why no statistically significant differences were observed in denture coverage among edentulous individuals across different financial groups in both the PolSenior and PolSenior2 studies.

However, partial dentures are only reimbursed for individuals with more than 5 missing teeth per jaw. This limitation may be a key factor contributing to the observed differences in denture coverage among partially edentulous individuals with varying financial statuses. The findings highlight the potential need for extending public reimbursement policies to include partially dentate individuals, which could help reduce financial barriers and improve access to dental prosthetics for a broader segment of the aging population.

Additionally, differences in denture use among individuals with varying financial backgrounds may be influenced by factors that extend beyond affordability, such as varying degrees of awareness, social engagement and overall lifestyle. Seniors in better financial situation may have greater access to dental care follow-ups, adjustments and maintenance, which can result in higher rates of denture use. These results emphasize the importance of considering socioeconomic factors when designing policies aimed at enhancing oral health care accessibility and utilization among aging populations.

Limitations

The present study relies on self-reported data, which may be subject to recall bias or reporting inaccuracies. Additionally, while the comparison between PolSenior and PolSenior2 offers valuable insights into trends in unmet denture needs, it does not reflect more recent developments in dental service accessibility or account for policy changes introduced after 2019. Future studies could benefit from the collection of longitudinal data and the inclusion of qualitative insights to better understand the individual motivations and barriers associated with denture coverage.

Conclusions

A comparative analysis of the PolSenior and PolSenior2 surveys demonstrated a significant reduction in unmet denture needs among older Polish adults over a 10-year period. This decline can be attributed to improved access to dental care and increased awareness of the significance of oral health within this population. The study highlights ongoing disparities in prosthetic coverage based on sex, age, place of residence, and education level, indicating areas where public health interventions could be further optimized. Enhancing access to dental care, especially in rural regions and among individuals with limited educational attainment, remains crucial. The high rates of denture utilization documented in both surveys suggest that these devices effectively address the functional and aesthetic needs of older adults. However, the observed decline in utilization among the oldest age groups highlights the need for additional support, such as tailored education on proper denture maintenance and increased access to follow-up dental care. It is imperative that ongoing research be conducted to monitor these trends and identify any additional factors that can further enhance the quality of life and well-being of Poland's aging population.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Wąsacz K, Chomyszyn-Gajewska M, Hukowska D. Oral health-related quality of life (OHRQoL) in Polish adults with periodontal diseases, oral mucosal diseases, and dental caries. *Dent Med Probl*. 2022;59(4):573–581. doi:10.17219/dmp/146195
2. Jain N, Dutt U, Radenkov I, Jain S. WHO's global oral health status report 2022: Actions, discussion, and implementation. *Oral Dis*. 2024;30(2):73–79. doi:10.1111/odi.14516
3. Lee KY, Chan CCK, Yip C, et al. Association between tooth loss-related speech and psychosocial impairment with cognitive function: A pilot study in Hong Kong's older population. *J Oral Rehabil*. 2024;51(8):1475–1485. doi:10.1111/joor.13718
4. Nakamura H, Noguchi-Shinohara M, Ishimiya-Jokaji M, et al. Brain atrophy in normal older adult links tooth loss and diet changes to future cognitive decline. *NPJ Aging*. 2024;10(1):20. doi:10.1038/s41514-024-00146-4
5. Petersen PE, Ogawa H. Promoting oral health and quality of life of older people – the need for public health action. *Oral Health Prev Dent*. 2018;16(2):113–124. doi:10.3290/j.ohpd.a40309
6. Qi X, Pei Y, Wang K, Han S, Wu B. Social isolation, loneliness, and accelerated tooth loss among Chinese older adults: A longitudinal study. *Community Dent Oral Epidemiol*. 2023;51(2):201–210. doi:10.1111/cdoe.12727
7. Starr JM, Hall RJ, Macintyre S, Deary IJ, Whalley LJ. Predictors and correlates of edentulism in the healthy old people in Edinburgh (HOPE) study. *Gerodontology*. 2008;25(4):199–204. doi:10.1111/j.1741-2358.2008.00227.x
8. Bourgeois D, Nihtila A, Mersel A. Prevalence of caries and edentulousness among 65–74-year-olds in Europe. *Bull World Health Organ*. 1998;76(4):413–417. PMID:9803592.
9. Dąbrowski W, Jagiełło K, Mossakowska M, et al. Evaluating changes in dental status among Polish older adults over a decade: A comparative analysis of PolSenior (2009) and PolSenior2 (2019) surveys. *Dent Med Probl*. 2025;62(1):23–30. doi:10.17219/dmp/196535
10. Piampring P. Problems with complete dentures and related factors in patients in Rajavithi Hospital from 2007 to 2012. *J Med Assoc Thai*. 2016;99 Suppl 2:S182–S187. PMID:27266234.
11. Suzuki H, Kanazawa M, Komagamine Y, et al. The effect of new complete denture fabrication and simplified dietary advice on nutrient intake and masticatory function of edentulous elderly: A randomized-controlled trial. *Clin Nutr*. 2018;37(5):1441–1447. doi:10.1016/j.clnu.2017.07.022
12. Suzuki H, Kanazawa M, Komagamine Y, Iwaki M, Amagai N, Minakuchi S. Changes in the nutritional statuses of edentulous elderly patients after new denture fabrication with and without providing simple dietary advice. *J Prosthodont Res*. 2019;63(3):288–292. doi:10.1016/j.jpor.2018.12.010
13. Campos Sugio CY, Mosquim V, Jacomine JC, et al. Impact of rehabilitation with removable complete or partial dentures on masticatory efficiency and quality of life: A cross-sectional mapping study. *J Prosthet Dent*. 2022;128(6):1295–1302. doi:10.1016/j.prosdent.2021.02.035
14. Hajek A, König HH. The association between oral health-related quality of life, loneliness, perceived and objective social isolation – results of a nationally representative survey. *Int J Environ Res Public Health*. 2021;18(24):12886. doi:10.3390/ijerph182412886
15. Muhammad T, Srivastava S. Tooth loss and associated self-rated health and psychological and subjective wellbeing among community-dwelling older adults: A cross-sectional study in India. *BMC Public Health*. 2022;22(1):7. doi:10.1186/s12889-021-12457-2
16. Kołciuk L, Godlewski T. Oral health-related quality of life of patients using removable dentures – review of literature. *Dent Med Probl*. 2015;52(2):222–226. https://dmp.umw.edu.pl/en/article/2015/52/2/222. Accessed March 12, 2025.

17. Wierucki Ł, Kujawska-Danecka H, Mossakowska M, et al. Health status and its socio-economic covariates in the older population in Poland – the assumptions and methods of the nationwide, cross-sectional PolSenior2 survey. *Arch Med Sci*. 2020;18(1):92–102. doi:10.5114/aoms.2020.100898
18. Błędowski P, Mossakowska M, Chudek J, et al. Medical, psychological and socioeconomic aspects of aging in Poland: Assumptions and objectives of the PolSenior project. *Exp Gerontol*. 2011;46(12):1003–1009. doi:10.1016/j.exger.2011.09.006
19. Altaie SF. Tribological, microhardness and color stability properties of a heat-cured acrylic resin denture base after reinforcement with different types of nanofiller particles. *Dent Med Probl*. 2023;60(2):295–302. doi:10.17219/dmp/137611
20. Listl S. Denture wearing by individuals among the older segment of European populations. *Int J Prosthodont*. 2012;25(1):15–20. PMID:22259790.
21. Zitzmann NU, Hagmann E, Weiger R. What is the prevalence of various types of prosthetic dental restorations in Europe? *Clin Oral Implants Res*. 2007;18 Suppl 3:20–33. doi:10.1111/j.1600-0501.2007.01435.x
22. Henriksen BM, Axéll T, Laake K. Geographic differences in tooth loss and denture-wearing among the elderly in Norway. *Community Dent Oral Epidemiol*. 2003;31(6):403–411. doi:10.1046/j.1600-0528.2003.00047.x
23. Yuan L, Yuan Y, Ren H, et al. Decomposition analysis of the prevalence of denture use between rural and urban edentulous elderly individuals in China: Cross-sectional study. *Interact J Med Res*. 2024;13:e48778. doi:10.2196/48778
24. Mukatash Nimri GE, Jebreen SE. Oral condition, treatment needs and demands of geriatric denture wearers in three different Jordanian communities. *JRMS*. 2016;23(3):40–48. doi:10.12816/0029072
25. Thompson AE, Anisimowicz Y, Miedema B, Hogg W, Wodchis WP, Aubrey-Bassler K. The influence of gender and other patient characteristics on health care-seeking behaviour: A QUALICOPC study. *BMC Fam Pract*. 2016;17:38. doi:10.1186/s12875-016-0440-0
26. Pinkhasov RM, Wong J, Kashanian J, et al. Are men shortchanged on health? Perspective on health care utilization and health risk behavior in men and women in the United States. *Int J Clin Pract*. 2010;64(4):475–487. doi:10.1111/j.1742-1241.2009.02290.x
27. Huraib WM, Al-Ghalib TA, Niyazi AAT, Bamigdad MS. Assessment of nutritional and psychosocial status of elderly patients wearing removable dental prosthetics. *J Pharm Bioallied Sci*. 2022;14(Suppl 1):S429–S432. doi:10.4103/jpbs.jpbs_840_21
28. Voinea-Griffin A, Solomon ES. Dentist shortage: An analysis of dentists, practices, and populations in the underserved areas. *J Public Health Dent*. 2016;76(4):314–319. doi:10.1111/jphd.12157
29. Northridge ME, Kumar A, Kaur R. Disparities in access to oral health care. *Annu Rev Public Health*. 2020;41:513–535. doi:10.1146/annurev-publhealth-040119-094318
30. McGrath C, Bedi R, Dhawan N. Factors influencing older people's self-reported use of dental services in the UK. *Gerodontology*. 1999;16(2):97–102. doi:10.1111/j.1741-2358.1999.00097.x
31. Ohi T, Sai M, Kikuchi M, et al. Determinants of the utilization of dental services in a community-dwelling elderly Japanese population. *Tohoku J Exp Med*. 2009;218(3):241–249. doi:10.1620/tjem.218.241

Original paper

Comparative effectiveness of silver diamine fluoride, papain-based material and CeraBur® in remineralizing or selectively removing infected dentin in primary teeth: A three-month randomized clinical trial

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Abstract

Background. The conventional method of removing caries lesions is an anxiety-inducing process that often necessitates the administration of local anesthesia and the extensive removal of tooth structure. Therefore, minimally invasive procedures are required to preserve tooth structure and minimize discomfort.

Objectives. The aim of the study was to compare 3 minimally invasive treatments for the management of dental caries, evaluating factors such as treatment time, the need for anesthesia, reported adverse events, and restorative material follow-up.

Material and methods. A clinical trial was conducted on 45 children aged 7–8 years with active caries in primary molars. The samples were divided into 3 groups based on the applied treatment: 38% silver diamine fluoride (e-SDF®) group; BRIX3000® group; and CeraBur® group. The duration of treatment was recorded using a stopwatch. Adverse events, including tooth pain irritations, lesions, spots, and discolorations, were reported by parents within 2 weeks. The durability of the restorative material, namely glass ionomer cement (GIC), was assessed after 3 months. The χ^2 and Kruskal–Wallis tests were conducted to analyze the data. The values were considered statistically significant at $p \leq 0.05$.

Results. A significant difference in the duration of caries removal procedure was noted between the 3 treatment methods based on the median values (e-SDF®: 471 s, CeraBur®: 171 s, BRIX3000®: 1,173 s) ($p < 0.001$). Post hoc pairwise comparisons indicated significant differences in duration of the procedure between the CeraBur® and BRIX3000® groups, as well as between the e-SDF® and BRIX3000® groups ($p < 0.001$). The need for local anesthesia varied significantly between the 3 methods ($p = 0.021$). A significant difference was observed in the incidence of postoperative complications among the 3 methods, with the rate of adverse events equaling 9 (60.0%) in the e-SDF® group and 2 (13.3%) in the remaining groups ($p = 0.013$). A 3-month follow-up revealed a borderline significant difference in GIC integrity among the 3 methods ($p = 0.052$).

Conclusions. Caries excavation using BRIX3000® required a longer duration compared with e-SDF® and CeraBur®. Silver diamine fluoride was found to be a simple, minimally invasive approach for caries management and was effective in reducing the need for local anesthesia during treatment. Further research is required to evaluate patient satisfaction and restoration outcomes over longer follow-up periods.

Keywords: silver diamine fluoride, ceramic bur, chemomechanical caries removal, minimally invasive dentistry, BRIX3000

Highlights

- CeraBur® achieved the shortest caries removal time (*Me* ~3 min), whereas BRIX3000® required substantially longer clinical time (*Me* ~19 min), which may influence chairside management.
- Silver diamine fluoride (e-SDF®) emerged as a simple, minimally invasive approach that effectively minimizes the need for local anesthesia in pediatric patients.
- Despite its clinical simplicity, e-SDF® was associated with tooth discoloration in approx. 60.0% of cases, compared with a 13.3% incidence of adverse events in the other treatment groups.
- At the 3-month follow-up, the integrity of glass ionomer cement (GIC) restorations did not differ significantly among the excavation methods used.

Introduction

Dental caries is a common condition that affects individuals on a global scale.[1] It occurs over an extended period and results from 3 factors, namely acid-producing bacteria, fermentable carbohydrates and host components, such as teeth and saliva.[2] Despite a decrease in the occurrence of caries in many industrialized nations as compared to previous decades, it remains an important public health problem.[3]

The conventional method of caries removal using the dental burs is the most prevalent procedure in dental treatment. Nevertheless, this technique is consistently linked to numerous drawbacks, including patients perceiving drilling as an unpleasant sensation, the frequent need for local anesthesia, the potential for drilling to cause harmful thermal effects on the pulp, and the possibility of excessive removal of the healthy tooth structure.[4]

The necessity for minimal intervention and painless dentistry, which provide relief and solace while fostering a positive attitude toward dental procedures, is one of the justifications for the field of pediatric dentistry.[5]

The objective of minimally invasive procedures is to preserve as much tooth structure as possible, to ensure that teeth remain functional for longer periods, and, in the case of deciduous teeth, to promote their retention until natural exfoliation. These methods have been shown to be both cost-effective and acceptable to parents and pediatric patients, while causing the least possible pain and discomfort to the child.[6]

Various strategies have been developed for the management of caries without the use of rotary devices, including mechanical atraumatic restorative treatment (ART), fluoride-based caries arrest, and other minimally invasive treatments such as chemomechanical caries removal (CMCR).[7]

For the elimination of carious dentin, a novel slow-speed rotary ceramic bur (CeraBur®) was constructed from zirconia stabilized with alumina–yttria. This instrument effectively eliminates caries from infected soft dentin and reduces the need for the spoon excavator.[8] Ceramic burs are advantageous for the excavation of dentin caries due to their superior cutting efficacy. Additionally, they possess an exceptionally efficient capacity for excavating delicate carious dentin while minimizing damage to the hard structure of the tooth. As a result, ceramic burs ought to be appropriate for minimally invasive caries excavation, as they result in a reduced number of dentinal tubules, thereby eliciting fewer pain sensations.[9]

According to Abdellatif et al., although fluoride varnish has shown efficacy in reducing caries, it lacks the capacity to cure deep carious lesions.[10] The efficacy of silver diamine fluoride (SDF) in halting caries progression in enamel lesions has been acknowledged.[11]

Silver diamine fluoride is considered a simple and cost-effective technique that does not require patient participation or complex training from healthcare providers. In resource-constrained regions, this approach may be very beneficial as a substitute for costly preventative interventions.[12]

The combined effects of fluoride's ability to facilitate remineralization and silver's antibacterial and collagenase inhibitor properties are synergistically used to impede the advancement of dental caries lesions and mitigate the occurrence of dental caries.[13]

The use of minimally invasive procedures has shown a noticeable rise, particularly in the context of pediatric patients. Chemomechanical caries removal is a minimally invasive approach that effectively disintegrates decaying tissue while conserving the integrity of the tooth structure and minimizing potential pulp irritation and patient distress. The method entails the removal of deteriorated tissue by using natural or synthetic substances to dissolve and facilitate the elimination of the diseased tissue.[7] The procedure requires the use of chemical agents to soften the carious dentin, which is then eliminated by a meticulous excavation process.[14] Chemomechanical agents for caries elimination have been utilized since 1975. Examples of such compositions include sodium hypochlorite,[15] GK-101[16] and Carisolv (Medi Team, Sävedalen, Sweden).[17]

The papain gel (Papacárie; Fórmula & Ação, São Paulo, Brazil) was developed as a result of a research initiative conducted in Brazil in 2003. The Carie-Care™ (UnibioTech Pharmaceuticals Pvt Ltd., Chennai, India), a gel produced from *Carica papaya* containing a purified enzyme, was created in India. This gel incorporates the antibacterial and analgesic properties of clove oil.[18]

BRIX3000® (Brix Medical Science), a chemomechanical agent, was introduced in 2012. It is a papain-based preparation, containing a proteolytic enzyme derived from the latex and fruits of green papaya (*C. papaya*). This enzyme functions as a chemical degradant. According to the producers, the distinguishing factor of this product is the quantity of papain used, specifically 3,000 U/mg at a concentration of 10%.[19]

Both SDF and BRIX3000® therapies are minimally invasive and effective in the management of early caries. However, a comparison of their working time, follow-up acceptance and overlying restoration durability remains to be conducted. The paucity of robust comparative studies has resulted in a limited clinical guidance on the optimal or most practical treatment of different patient populations, particularly with regard to safety, patient compliance and cost-effectiveness.

The present study was conducted to compare 3 minimally invasive treatments with regard to treatment time (efficiency), the need for anesthesia, reported adverse events, and restorative material (glass ionomer cement (GIC)) follow-up.

The null hypothesis stated that there was no discernible difference in terms of treatment time, reported adverse events and restoration integrity among the 3 substances: SDF (e-SDF®); a papain-based CMCR product (BRIX3000®); and a ceramic bur (CeraBur®).

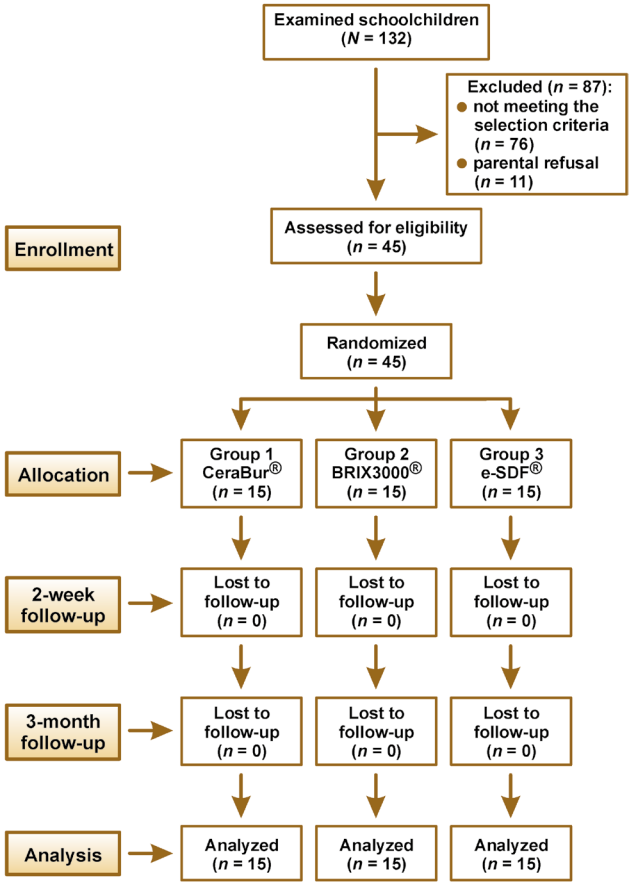

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study

SDF – silver diamine fluoride.

Material and methods

A randomized clinical trial was conducted on a sample of schoolchildren who were visiting the postgraduate clinic at the Department of Orthodontics, Pedodontics and Preventive Dentistry, College of Dentistry, Mustansiriyah University (Baghdad, Iraq). The trial was conducted following the Consolidated Standards of Reporting Trials (CONSORT) checklist. The process of collecting data commenced in early December 2023 and continued until April 2024.

Study sample

The study population comprised 45 children, selected from a total of 132 children attending second stage of primary school. Eighty-seven subjects were excluded due to either non-compliance with the established criteria or due to their parents' refusal (Fig. 1). The sample size was determined according to the previous studies[20,21] regarding GIC durability, secondary caries and pathological change after 3 months of recall. The sample size was double-checked using G*Power v. 3.1.9.4 software (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany). With the effect size (*f*) of 0.5, the α error probability of 0.05, the power (1–β error probability) of 0.80, and the actual power of 0.8034136, the sample size was determined to be 42. The size of the study group was increased to 45 participants in order to account for possible dropouts.

A total of 45 participants were randomly divided into 3 groups of 15 participants each (allocation ratio of 1:1:1), as follows: e-SDF® group; BRIX3000® group; and CeraBur® group. The randomization code was generated using Research Randomizer software (https://www.randomizer.org). A block size of 3 was used to ensure equal numbers of patients in all 3 groups. The allocation of patients to treatment groups was concealed using sealed envelope techniques.

The study participants were aged between 7 and 8 years and had active caries lesions on their primary molars (International Caries Detection and Assessment System (ICDAS) II code 5). The subjects were selected based on particular criteria.

Selection criteria

Children were included in the current investigation provided they met the following criteria, as reported by multiple studies,[22–24] with some modifications:

1. The children had no documented history of oral or systemic disorders, and were in good physical condition.

2. Each child presented with primary molars with open carious lesions, either on the occlusal surface or the proximal surface, in the absence of a neighboring tooth (slot cavity). These lesions affected the dentin but did not expose the pulp. The depth of the cavities was measured using a DIAGNOdent caries detector, with a range of 40–99, in accordance with the ICDAS II code 5, which does not involve the pulp.[25]
3. The cavities were easily reachable by hand tools and were of a size sufficient to allow the entry of a small excavator.
4. There was no clinical evidence of pulp or periapical infections in the vital primary molars, and the patient was not exhibiting any symptoms.
5. The teeth with a normal cusp and fossa, free from attrition, destruction or enamel defects that affect the retention of GIC restoration were chosen.
6. The child's behavior was deemed as accurate when assessed using Wright's clinical classification[26] as "cooperative", excluding both "lacking in cooperative ability" and "potentially cooperative" categories.

Study groups

The study sample was divided into 3 groups, as follows:
- e-SDF® group: treated with 38% SDF (e-SDF®; Kids-e-dental LLP, Mumbai, India);
- BRIX3000® group: treated with a papain-based gel (BRIX3000®; OralMed Global LTD, Carcarañá, Argentina);
- CeraBur® group: control group treated with a slow-speed handpiece with a ceramic bur (CeraBur® K1SM; Komet, Lemgo, Germany).

Participants were randomly assigned to one of three groups and received a specific treatment technique for a designated tooth.

Oral examination method

The oral examination was conducted after the child was positioned in the dentist's chair, with the operation light providing assistance. In order to maintain the consistency and validity of the study, the study procedure was accomplished using appropriate equipment by a single operator with 5 years of experience in the field, who had undergone dedicated training. The identification of dental caries was performed using visual and tactile perceptions, with the aid of a dental mirror and a probe. Before the initiation of the caries removal procedure, the depth of carious lesions was evaluated using the ICDAS II and the DIAGNOdent caries detector that was calibrated before each patient. The research process continued when the subject met all the specified requirements.

Clinical procedure

The clinical procedure was conducted according to the manufacturer's guidelines and instruction catalogue for each material used.[21,27–29]

All teeth were isolated using a cotton roll and a saliva ejector. The occlusal surface was brushed to remove any accumulated debris and plaque. Before the commencement of caries removal treatment, the depth of carious lesions was evaluated using the DIAGNOdent caries detector, and the findings were documented in the patient's case sheet. The measurement of time was initiated with the use of a stopwatch.

In the BRIX3000® group, the manufacturer's guidelines were followed; BRIX3000® was applied to the cavity using a microbrush and left in place for approx. 2–3 min. Subsequently, the infected dentin that had dissolved was removed with a spoon excavator, without applying pressure or making incisions. In instances where the cavity was still occupied with infected dentin, the implementation of an additional layer of the agent might have been necessary (Fig. 2). When sound dentin of the cavity was reached and all infected dentin was removed (Fig. 3), the stopwatch was halted and the duration of the procedure was recorded in the case sheet, signifying the completion of the caries removal process.

In the e-SDF® group, patients' faces and gums were protected with a petroleum gel (Vaseline®) to avoid staining.

Fig. 2. Application of BRIX3000® to the cavity of the lower left first primary molar using a microbrush, after which the agent was left in place for approx. 2–3 min to dissolve the carious lesion

Fig. 3. Sound dentin after excavation of the dissolved active lesion using a blunt instrument

The application of 38% SDF was performed twice with a microbrush on the affected tooth surface, and surplus material was removed using cotton pellets (Fig. 4). In accordance with the manufacturer's instructions, the time required for the material to act is 2 min per single application. The black discoloration manifested subsequent to double application of the agent (Fig. 5), after which the stopwatch was halted and the duration of the procedure was recorded in the case sheet, signifying the conclusion of caries treatment.

In the CeraBur® group, caries removal was performed using a low-speed handpiece with a ceramic bur (CeraBur® K1SM; Komet). In the absence of a water coolant, the removal of carious tissue was conducted through the use of circular motions, from the center of the cavity to its boundary. Following the identification of hard dentin, the process of caries excavation was stopped, the stopwatch was halted, and the duration of the procedure was recorded in the case sheet, thus signifying the completion of the caries removal process.

The tooth surface in all 3 treatment groups was conditioned with polyacrylic acid to remove the smear layer. Subsequently, it was washed and dried using dry pellets.

The glass ionomer restoration (Riva Self Cure; SDI Inc., Bayswater, Australia) was prepared according to the manufacturer's instructions. The mixed glass ionomer was placed into the cavity, with the applier's flat end being utilized to ensure the material was introduced into the corners of the cavity, resulting in slight overfilling. The bite was checked and any excess material was manually extracted, if necessary. The patient was instructed to refrain from eating for at least 1 h.

Fig. 4. Cavity preparation and debris removal prior to the application of 38% silver diamine fluoride (e-SDF®) to the lower right second primary molar

Fig. 5. Black discoloration of infected dentin after double application of 38% silver diamine fluoride (e-SDF®)

Time assessment

The duration of therapy for each group was measured using a digital stopwatch. The stopwatch for all study groups was started as soon as the depth assessment was recorded by the DIAGNOdent.

In the CeraBur® and BRIX3000® groups, the time was stopped as the cavity was found to be caries-free. In the e-SDF® group, the time for the black discoloration to manifest after double application of e-SDF® was 2 min, as determined by the manufacturer's instructions.

In cases where local anesthesia was necessary, the stated time for caries removal included the time allocated for the administration of the anesthetic agent.

Adverse events

The incidence of potential adverse events in 3 groups reported by the patients within 2 weeks was the primary outcome of the study. Complications include tooth pain or sensitivity, poor taste, tooth discoloration, and potential irritations, lesions, or spots on the mucosa, gingiva and skin.

Restoration follow-up

Recall and follow-up examinations were performed at 3 months and regarded as a secondary outcome of the study. The assessment ratings were adapted from the study by Satyarup et al.[28] and scored as follows: score 1 – restoration is fully intact, covering all pits and fissures; score 2 – restoration is partially lost, but the tooth itself is in good condition with no active or soft caries; score 3 – restoration is partially lost and the tooth has active or soft caries; score 4 – restoration is completely lost, but the tooth itself is sound; score 5 – restoration is completely lost and the tooth has caries. A tooth was deemed sound if its surface exhibited a firm and lustrous texture.[28]

Statistical analysis

The data analysis was conducted using the IBM SPSS Statistics for Windows software, v. 26.0 (IBM Corp., Armonk, USA). The χ^2 test was utilized to compare the percentages of nominal variables between two or more independent groups. The Kruskal–Wallis test was performed to test hypotheses concerning medians of quantitative and ordinal variables between more than 2 independent groups. Values were considered statistically significant at $p \leq 0.05$.

Results

The sample distribution according to age, sex, type of teeth treated, and cavity location is illustrated in Tables 1 and 2.

Table 1. Demographic and clinical characteristics of the study sample

Variable		*n* (%)
Age	7 years	15 (33.33)
	8 years	30 (66.66)
Sex	male	23 (51.11)
	female	22 (48.88)
Tooth number (FDI numbering system)	54	6 (13.3)
	55	5 (11.1)
	64	3 (6.7)
	65	2 (4.4)
	74	11 (24.4)
	75	7 (15.6)
	84	8 (17.8)
	85	3 (6.7)
Selected cavity location	proximal	22 (48.9)
	occlusal	23 (51.1)
Total		45 (100.0)

FDI – World Dental Federation.

Table 2. Distribution of tooth type according to study group

Group	Tooth number (FDI numbering system), *n*								Total, *n*
	54	55	64	65	74	75	84	85	
e-SDF®	2	1	1	0	6	2	2	1	15
CeraBur®	3	3	1	1	1	1	3	2	15
BRIX3000®	1	1	1	1	4	4	3	0	15

SDF – silver diamine fluoride.

Duration of caries removal procedure

As demonstrated in Table 3, a significant difference in the duration of caries removal was observed among the 3 treatment methods ($p < 0.001$). Post hoc pairwise comparisons indicated significant differences between the CeraBur® and BRIX3000® groups ($p < 0.001$), as well as between the e-SDF® and BRIX3000® groups ($p < 0.001$) (Table 4).

Table 3. Duration of caries removal treatment in the 3 study groups

Variable	Group	*n*	*Me* (*IQR*)	*p*-value
Time [s]	e-SDF®	15	471 (66)	<0.001*
	CeraBur®	15	171 (309)	
	BRIX3000®	15	1,173 (186)	

* statistically significant ($p \leq 0.05$, Kruskal–Wallis test); *Me* – median; *IQR* – interquartile range.

Table 4. Post hoc pairwise comparisons of caries removal duration among the 3 study groups

Groups	*p*-value
e-SDF® × CeraBur®	0.203
CeraBur® × BRIX3000®	<0.001*
e-SDF® × BRIX3000®	<0.001*

* statistically significant ($p \leq 0.05$).

Need for anesthesia

Table 5 shows a significant difference in the requirement for anesthesia among the 3 caries treatment methods ($p = 0.021$). Specifically, 4 out of 15 children treated with the CeraBur® method required dental anesthesia during the caries removal procedure. In contrast, none of the children treated with e-SDF® or BRIX3000® required anesthesia.

Table 5. Requirement for anesthesia during the clinical procedure according to treatment method

Group	Yes, *n* (%)	No, *n* (%)	*p*-value
e-SDF®	0 (0.0)	15 (100.0)	0.021*
CeraBur®	4 (26.7)	11 (73.3)	
BRIX3000®	0 (0.0)	15 (100.0)	

* statistically significant ($p \leq 0.05$, χ^2 test).

Postoperative adverse events

The study outcomes revealed a significant difference in postoperative adverse events among the 3 treatment methods ($p = 0.013$). Specifically, 9 out of 15 children treated with the SDF method reported a discoloration following the clinical procedure of caries removal, compared to only 2 of the children treated with the CeraBur® or BRIX3000® methods who showed dental sensitivity or pain (Table 6).

Table 6. Prevalence of postoperative adverse events according to treatment method

Group	Yes, *n* (%)	No, *n* (%)	*p*-value
e-SDF®	9 (60.0)	6 (40.0)	0.013*
CeraBur®	2 (13.3)	13 (86.7)	
BRIX3000®	2 (13.3)	13 (86.7)	

* statistically significant ($p \leq 0.05$, χ^2 test).

GIC filling after 3 months

As illustrated in Table 7, a non-significant difference in GIC filling integrity is evident among the 3 caries treatment methods following 3 months of follow-up ($p = 0.052$).

Table 7. Integrity of glass ionomer restorations in the 3 treatment groups after 3 months, assessed using scoring criteria adapted from Satyarup et al.[28]

Group	*n*	Restoration integrity score *Me* (*IQR*)	*p*-value (Kruskal–Wallis test)
e-SDF®	15	1 (1)	
CeraBur®	15	2 (1)	0.052
BRIX3000®	15	2 (1)	

score 1 – restoration is fully intact, covering all pits and fissures; score 2 – restoration is partially lost, but the tooth itself is in good condition with no active or soft caries; score 3 – restoration is partially lost and the tooth has active or soft caries; score 4 – restoration is completely lost, but the tooth itself is sound; score 5 – restoration is completely lost and the tooth has caries.

Discussion

Conventional caries removal methods involve drilling with a high-speed handpiece to access the carious lesions and a low-speed handpiece to remove the carious tissue. Although fast and efficient caries removal can be achieved using these techniques, drilling stimulates discomfort and pain. Therefore, local anesthesia is routinely needed.[22] The employment of minimally invasive techniques can address and overcome other issues, such as overheating the pulp, vibration and noise.

In the present study, the examination of dental caries was performed for each tooth to determine the depth of the carious lesion. The DIAGNOdent caries detection instrument was used in numerous studies as a non-invasive method of assessing the extent of lesions.[30,31] While its sensitivity and specificity have been accepted, it should be used in combination with a visual method to minimize false positive errors.[32] Thus, in the present study, the ICDAS II code 5, which does not involve the pulp for occlusal and proximal lesions, was used in addition to the DIAGNOdent to evaluate lesion depth.[25]

In pediatric dentistry, it is important to reduce procedure time, ensure pain-free intervention, and opt for restorations that are less technique-sensitive to manage the child's behavior. In the course of the present study, the use of SDF did not elicit a pain reaction, as this method does not necessitate the application of pressure to caries lesions. Furthermore, the CMCR technique requires minimum pressure to remove softened caries tissue from the tooth cavity. As a result, both of these groups had a significantly lower demand for anesthesia in comparison to the CeraBur® group.

Although a ceramic bur removes infected dentin only, it produces mild vibrations during the process of cavity preparation. Dental anesthesia was applied according to the patient's pain threshold and to control the child's behavior. This result was consistent with the studies by Nagaveni et al.[5] and Ismail and Haidar,[22] with the latter concluding that BRIX3000® removes infected dentin only, thus eliminating the painful removal of sound dentin or the need for local anesthesia.

In pediatric patients, it is important to balance between the duration of the procedure and the efficient management of behavior.[33] Previous studies have suggested a relationship between behavior during treatment and treatment duration in children.[34] Consequently, when developing a therapy plan for pediatric patients, chair time is a crucial consideration, as longer treatments can trigger negative behaviors in children. Furthermore, it is well recognized that shorter treatment durations result in lower expenses and an increased number of patients receiving benefits, particularly in the field of public health.[35] The time of the procedure in the dental clinic depends on multiple factors, including child behavior, procedure acceptance, controlling tooth isolation, the presence of debris on the available carious cavity, the time required for cavity preparation, and the dentist's experience. In the current study, the time was measured in each group excluding any sudden intervals in the procedure that were out of procedural focus. Regarding treatment efficiency, the BRIX3000® group required the longest treatment time, likely due to variation in lesion consistency (soft, medium, or hard), which necessitated repeated applications of the BRIX3000® gel, most commonly 2–3 times per case, followed by mechanical excavation of the infected dentin. In some cases, conventional drills were used to get access to the lesion in the presence of undermined enamel. A ceramic bur is composed of alumina–yttria ceramic, which exhibits excellent wear resistance and cutting ability. Multiple studies have demonstrated that this material allows faster caries removal compared with CMCR.[22,29,36] Therefore, the CMCR method may be associated with higher levels of anxiety, as it requires a longer treatment duration.[37] Although the SDF method was more time-consuming than the CeraBur® technique, the latter requires the administration of dental anesthesia, which contributes substantially to the overall treatment time (Fig. 6) and may negatively influence the child behavior. In contrast, the SDF method demonstrated a shorter duration than the CMCR procedure, as it involved tooth cleaning and manufacturer-standardized application times. Treatment duration was dependent on the number of applications required, with a maximum of 2 applications per tooth. These findings are consistent with those reported by Vollú et al.[35]

The adverse events recorded in the present study included discoloration of the teeth and gingiva, pain and tooth sensitivity during the 2-week follow-up, as reported by the parents. As has been demonstrated in a number of studies,[35,38,39] discoloration was identified as an adverse event in the majority of cases. However, in 1 study, this was not considered to be the case, and the reported side effects were either diarrhea or stomachache after SDF treatment.[40]

Regarding that adverse events were significantly more prevalent in the e-SDF® group, they included only discoloration of gingival tissues that resolved within 2 days,

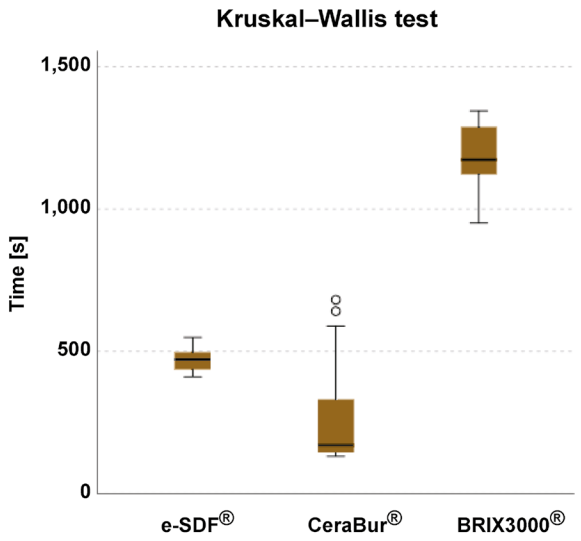

Fig. 6. Box plot presenting the time required to perform treatment in the 3 groups

During the administration of dental anesthesia in the CeraBur® group, 2 outlier patients caused an increase in the median treatment time beyond the range observed in the remaining 13 participants.

and black staining on the arrested lesions of teeth. The absence of pain and sensitivity was also noted. These outcomes are similar to those reported in the studies by Vollú et al., Fung et al. and Duangthip et al.[35,38,39] Despite the cosmetic effects of SDF, parents were informed about the effects of other treatment methods and were given information to help them make an informed choice and weigh up the advantages of SDF's non-invasiveness with the aesthetic concerns.

The remaining treatment groups (CeraBur® and BRIX3000®) exhibited pain and sensitivity in 13% of cases. Although these symptoms do not necessarily indicate serious or irreversible damage, they may still have clinical relevance. Moreover, parental reports of children's symptoms may be subject to misinterpretation, either underestimating or overestimating their severity and duration, which may range from transient to more persistent. Although these 2 groups show relatively low frequency of adverse events, the presence of pain and sensitivity may be associated with residual caries and can negatively affect the child's quality of life, representing a more significant concern than discoloration alone. In this context, despite the undesirable dark staining associated with SDF, its benefits (such as caries arrest and non-invasiveness) are considered to outweigh the cosmetic drawbacks.[41]

Numerous minimally invasive procedures, including ART, involve hand instrument-based excavation of carious tissue followed by cavity restoration without the use of bonding agents. These approaches have demonstrated good biocompatibility, particularly when a cement is used, and high thermal expansion.[42] Nevertheless, the application of SDF results in tooth discoloration, potentially compromising aesthetic appeal. This highlights the need for restorative materials that effectively improve aesthetics while maintaining efficacy.[41] A tooth-colored GIC containing a high concentration of fluoride ions, which remineralize carious lesions and prevent further caries development, can also be utilized to arrest active caries.

The follow-up examinations were conducted at 3 months following the assessment rating adapted from the study by Satyarup et al.[28] The integrity of restorations post-treatment could be affected by the efficacy of caries removal, the extent of caries, cavity dimensions, tooth location, and the type of cavity, given that mastication affects restoration durability.

Although the e-SDF® group showed better retention of the GIC restoration, it can be attributed to the fluoride released from SDF, which enhances dentin resistance and reduces acid penetration.[43] Silver diamine fluoride has antibacterial and remineralizing properties that strengthen the marginal integrity, which is an important factor in the management of deep carious dentin.[44] The study results showed that the differences were borderline significant between the groups and that was in harmony with the studies by Raskin et al., Satyarup et al. and Zhi et al.[28,45,46] This dissimilarity may be attributed to the short-term 3-month follow-up, whereas the other studies implemented 6-month follow-ups for the evaluation of GIC restoration.

Riva Self Cure glass ionomer cement is a high-viscosity glass hybrid material that demonstrates a high clinical success rate and significantly reduced microleakage in Class II slot cavities, with performance comparable to that observed in Class I cavities.[47–49] In the course of our study, teeth with Class I and Class II slot preparations were evaluated. Based on these findings, high-viscosity GIC can be recommended as a reliable restorative option for Class I and Class II restorations in short-term follow-up. However, long-term clinical studies are required to further assess the survival rate of GICs in Class II cavities.[50]

A longer follow-up period would offer further explanation and could change the results regarding secondary caries, marginal mechanical wear or durability of GIC restoration.[51] However, there would be an increased risk of attrition and drop-out problems in follow-up among study cases.

The findings of the present study reveal that there is no statistically significant difference between the CeraBur® and BRIX3000® methods of caries removal based on restoration integrity. A study by Alkhawaja and Al Haidar showed that CeraBur® and BRIX3000® exhibited comparable performance in terms of microleakage of glass ionomer restoration. Both treatment groups focused on the removal of infected dentin, without exerting an effect on the remineralization of the lesion.[20] Another study concluded that there was no difference regarding residual dentin (smear layer, surface irregularities, intertubular microporosities, and exposed tubules) between smart burs and BRIX3000® after caries excavation, a process that affects the restorative material retention.[52]

Limitations

The sample of the study was limited to schoolchildren aged 7–8 years. This may constrain the generalizability of the findings to other populations. The study was conducted over a period of 3 months; longer follow-up periods are recommended to obtain a clearer idea about the retention and mechanical wear resistance of restorations. Certain outcomes, such as anxiety level, were not measured, as a separate study will be conducted on this aspect. The effectiveness of BRIX3000® and e-SDF® in reducing *Streptococcus mutans* count was not assessed. Further studies could explore the cost-effectiveness of SDF as opposed to other minimally invasive techniques.

Conclusions

Among the minimally invasive procedures evaluated in this study, the use of the ceramic bur resulted in the shortest treatment time. Caries excavation using BRIX3000® required a longer duration compared with both the ceramic bur and SDF application. The utilization of CMCR may be considered an alternative to conventional caries removal techniques, as it minimizes the need for local anesthesia. The only noticeable issue reported by parents regarding SDF application was tooth discoloration.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki. Ethics approval was granted by the Ethics Committee of the College of Dentistry of the Mustansiriyah University (Baghdad, Iraq) (No. MUPRU004). Prior to participation, parents or legal guardians were provided with comprehensive information about the study design, objectives and potential benefits. Written informed consent was obtained, and parents or guardians were informed of their right to withdraw their child from the study at any time.

Trial registration

The trial protocol was registered on ClinicalTrials.gov (registration ID: NCT06412731; https://clinicaltrials.gov/study/NCT06412731).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Al-Mashhadaney DMAK. Prevalence of dental caries in relation to residential factor among (6–9) years old children in Baghdad. *Mustansiria Dent J*. 2018;6(3):237–241. doi:10.32828/mdj.v6i3.464
2. Hasan DM, Abbas MJ, Al-Ghurabi, Hassan B. Impact of indium oxide nanoparticles mouth wash in prevention of human dental enamel caries (in vitro study). *Med J Babylon*. 2023;20(2):322–331. doi:10.4103/MJBL.MJBL_345_22
3. Basak SS, Dokumacioglu E. Evaluation of the effects of different mouthrinses on dental remineralization. *Dent Med Probl*. 2023;60(2):219–225. doi:10.17219/dmp/134290
4. Kotb RMS, Abdella AAH, El Kateb MA, Ahmed AM. Clinical evaluation of Papacarie in primary teeth. *J Clin Pediatr Dent*. 2009;34(2):117–123. doi:10.17796/jcpd.34.2.f312p36g18463716
5. Nagaveni NB, Radhika NB, Satisha TS, Ashwini KS, Neni S, Gupta S. Efficacy of new chemomechanical caries removal agent compared with conventional method in primary teeth: An in vivo study. *Int J Oral Health Sci*. 2016;6(2):52–58. doi:10.4103/2231-6027.199986
6. BaniHani A, Santamaría RM, Hu S, Maden M, Albadri S. Minimal intervention dentistry for managing carious lesions into dentine in primary teeth: An umbrella review. *Eur Arch Paediatr Dent*. 2022;23(5):667–693. doi:10.1007/s40368-021-00675-6
7. Júnior ZSS, Botta SB, Ana PA, et al. Effect of papain-based gel on type I collagen – spectroscopy applied for microstructural analysis. *Sci Rep*. 2015;5:11448. doi:10.1038/srep11448
8. Dammaschke T, Vesnic A, Schafer E. In vitro comparison of ceramic burs and conventional tungsten carbide bud burs in dentin caries excavation. *Quintessence Int*. 2008;39(6):495–499. PMID:19057746.
9. de Almeida Neves A, Coutinho E, Cardoso MV, Lambrechts P, Van Meerbeek B. Current concepts and techniques for caries excavation and adhesion to residual dentin. *J Adhes Dent*. 2011;13(1):7–22. doi:10.3290/j.jad.a18443
10. Abdellatif HM, Ali AM, Baghdady SI, ElKateb MA. Caries arrest effectiveness of silver diamine fluoride compared to alternative restorative technique: Randomized clinical trial. *Eur Arch Paediatr Dent*. 2021;22(4):575–585. doi:10.1007/s40368-020-00592-0
11. Crystal YO, Janal MN, Yim S, Nelson T. Teaching and utilization of silver diamine fluoride and Hall-style crowns in US pediatric dentistry residency programs. *J Am Dent Assoc*. 2020;151(10):755–763. doi:10.1016/j.adaj.2020.06.022
12. Abdulelah Abdulrahmn Almallah L. Topical effect of silver diamine fluoride in preventing and arresting root caries in elderly patients. *Mustansiria Dent J*. 2021;17(1):50–57. doi:10.32828/mdj.v17i1.1015
13. Mahmood Fahmi MQ, Abbas MJ, Abdulrahman A, Abdulelah L. Topical effect of silver diamine fluoride in arresting dental caries in primary teeth. *Indian J Public Health Res Dev*. 2019;10(5):1285. doi:10.5958/0976-5506.2019.01173.2
14. Mohammed TI, Salih SA. The effect of chemomechanical caries removal and different bonding systems on shear bond strength of carious dentin (in vitro study). *Mustansiria Dent J*. 2018;8(2):115–126. doi:10.32828/mdj.v8i2.314
15. Habib CM, Kronman J, Goldman M. A chemical evaluation of collagen and hydroxyproline after treatment with GK-101 (N-chloroglycine). *Pharmacol Ther Dent*. 1975;2(3–4):209–215. PMID:1073184.
16. Schutzbank SG, Galaini J, Kronman JH, Goldman M, Clark RE. A comparative in vitro study of GK-101 and GK-101E in caries removal. *J Dent Res*. 1978;57(9–10):861–864. doi:10.1177/00220345780570090201
17. Ericson D, Zimmerman M, Raber H, Götrick B, Bornstein R, Thorell J. Clinical evaluation of efficacy and safety of a new method for chemo-mechanical removal of caries. A multi-centre study. *Caries Res*. 1999;33(3):171–177. doi:10.1159/000016513

18. Hegde AM, Preethi VP, Shetty A, Shetty S. Clinical evaluation of chemo-bechanical caries removal using carie-care system among school children. *J Health Allied Sci NU*. 2014;04(03):080–084. doi:10.1055/s-0040-1703807
19. Maashi MS, Elkhodary HM, Alamoudi NM, Bamashmous NO. Chemomechanical caries removal methods: A literature review. *Saudi Dent J*. 2023;35(3):233–243. doi:10.1016/j.sdentj.2023.01.010
20. Alkhawaja HAA, Al Haidar, Aseel Haidar MJ. Microleakage evaluation of glass hybrid restoration following usage of papain-based gel and ceramic bur for caries removal: An in vitro study. *Dent Hypotheses*. 2022;13(2):57–60. doi:10.4103/denthyp.denthyp_48_22
21. Mahajan P, Pandit IK, Gugnani N, Gupta M. Comparative evaluation of 'less invasive approach' of caries excavation and 'only arrest and no excavation approach' in dental caries management in primary molars – A randomised controlled trial. *J Adv Med Dent Scie Res*. 2021;9(8):84–92. https://www.jamdsr.com/htmlissue.php?id=4250. Accessed January 10, 2024.
22. Ismail MMM, Haidar A. Evaluation of the efficacy of caries removal using papain gel (Brix 3000) and smart preparation bur (in vivo comparative study). *J Pharm Sci Res*. 2019;11(2):444–449. https://global-ie.com/wp-content/uploads/2022/02/comparativo-brix-vs-fresas-inteligentes.pdf. Accessed January 13, 2024.
23. Jiang M, Wong MCM, Chu CH, Dai L, Lo ECM. A 24-month randomized controlled trial on the success rates of restoring untreated and SDF-treated dentine caries lesions in primary teeth with the ART approach. *J Dent*. 2020;100:103435. doi:10.1016/j.jdent.2020.103435
24. Shivasharan P, Farhin A, Wakpanjar M, Shetty A. Clinical evaluation of caries removal in primary teeth using carie-care and SmartPrep burs: An in vivo study. *Indian J Oral Health Res*. 2016;2(1):27–31. doi:10.4103/2393-8692.184732
25. Dikmen B. Icdas II criteria (international caries detection and assessment system). *J Istanb Univ Fac Dent*. 2015;49(3):63–72. doi:10.17096/jiufd.38691
26. Dean JA, ed. *McDonald and Avery's Dentistry for the Child and Adolescent*. 11th ed. St. Louis, MI: Elsevier; 2021:346.
27. Ardeshana A, Karri A, Patel NG, Sura S. Evaluation of caries excavation efficacy with ceramic bur and hand excavation in primary teeth: An experimental study. *J South Asian Assoc Pediatr Dent*. 2020;3(2):60–64. doi:10.5005/jp-journals-10077-3054
28. Satyarup D, Mohanty S, Nagarajappa R, Mahapatra I, Dalai RP. Comparison of the effectiveness of 38% silver diamine fluoride and atraumatic restorative treatment for treating dental caries in a school setting: A randomized clinical trial. *Dent Med Probl*. 2022;59(2):217–223. doi:10.17219/dmp/143547
29. Elfeel NM, Aboelmaaty MM, Mostafa MH, El-Sayed SR. Evaluation of the efficacy of minimal invasive methods versus conventional methods for caries removal in primary molars. *Cureus*. 2023;15(12):e50803. doi:10.7759/cureus.50803
30. Bhat SS, Sain S, Hegde SK, Bhat V. Efficacy of chemomechanical caries removal. *Int J Appl Dent Sci*. 2015;1(3):27–30. https://www.oraljournal.com/archives/2015.v1.i3.A.27/efficacy-of-chemomechanical-caries-removal. Accessed October 2, 2024.
31. Alhuda DA, Rajab A, Shaadouh R, Katbeh I, Tuturov N, Arrag EA. Efficacy evaluation of pepsin enzyme-based gel in chemomechanical caries removal on primary anterior teeth: A randomized controlled clinical study. *Braz Dent Sci*. 2022;25(4):1–9. doi:10.4322/bds.2022.e3538
32. Nokhbatolfoghahaie H, Alikhasi M, Chiniforush N, Khoei F, Safavi N, Yaghoub Zadeh B. Evaluation of accuracy of DIAGNOdent in diagnosis of primary and secondary caries in comparison to conventional methods. *J Lasers Med Sci*. 2013;4(4):159–167. PMID:25606325.
33. Jamali Z, Najafpour E, Ebrahim Adhami Z, Sighari Deljavan A, Aminabadi NA, Shirazi S. Does the length of dental procedure influence children's behavior during and after treatment? A systematic review and critical appraisal. *J Dent Res Dent Clin Dent Prospects*. 2018;12(1):68–76. doi:10.15171/joddd.2018.011
34. Davidovich E, Wated A, Shapira J, Ram D. The influence of location of local anesthesia and complexity/duration of restorative treatment on children's behavior during dental treatment. *Pediatr Dent*. 2013;35(4):333–336. PMID:23930632.
35. Vollú AL, Rodrigues GF, Rougemount Teixeira RV, et al. Efficacy of 30% silver diamine fluoride compared to atraumatic restorative treatment on dentine caries arrestment in primary molars of preschool children: A 12-months parallel randomized controlled clinical trial. *J Dent*. 2019;88:103165. doi:10.1016/j.jdent.2019.07.003
36. Sharma N, Sisodia S, Jain A, Bhargava T, Kumar P, Rana KS. Evaluation of the efficacy of recent caries removal techniques: An in vitro study. *Cureus*. 2023;15(1):e34432. doi:10.7759/cureus.34432
37. Aminabadi NA, Oskouei SG, Farahani RMZ. Dental treatment duration as an indicator of the behavior of 3- to 9-year-old pediatric patients in clinical dental settings. *J Contemp Dent Pract*. 2009;10(5):E025–E032. PMID:19838607.
38. Fung MHT, Duangthip D, Wong MCM, Lo ECM, Chu CH. Randomized clinical trial of 12% and 38% silver diamine fluoride treatment. *J Dent Res*. 2018;97(2):171–178. doi:10.1177/0022034517728496
39. Duangthip D, Chu CH, Lo ECM. A randomized clinical trial on arresting dentine caries in preschool children by topical fluorides – 18 month results. *J Dent*. 2016;44:57–63. doi:10.1016/j.jdent.2015.05.006
40. Milgrom P, Horst JA, Ludwig S, et al. Topical silver diamine fluoride for dental caries arrest in preschool children: A randomized controlled trial and microbiological analysis of caries associated microbes and resistance gene expression. *J Dent*. 2018;68:72–78. doi:10.1016/j.jdent.2017.08.015
41. Crystal YO, Marghalani AA, Ureles SD, et al. Use of silver diamine fluoride for dental caries management in children and adolescents, including those with special health care needs. *Pediatr Dent*. 2017;39(5):135–145. PMID:29070149.
42. Jaber GS, Khashan KS, Abbas MJ, Ibrahim NA. Improvement of glass ionomer restoration by adding ZnO nanoparticles prepared by laser in (vitro study). *J Indian Chem Soc*. 2023;100(2):100879. doi:10.1016/j.jics.2023.100879
43. Rosenblatt A, Stamford TCM, Niederman R. Silver diamine fluoride: A caries "silver-fluoride bullet". *J Dent Res*. 2009;88(2):116–125. doi:10.1177/0022034508329406
44. Anani H, Elasser D, Niazy M, Jamil W, Elsharkawy D. Evaluation of the remineralization and antibacterial effect of natural versus synthetic materials on deep carious dentin: A randomized controlled trial. *Dent Med Probl*. 2023;60(1):87–97. doi:10.17219/dmp/147075
45. Raskin SE, Tranby EP, Ludwig S, Okunev I, Frantsve-Hawley J, Boynes S. Survival of silver diamine fluoride among patients treated in community dental clinics: A naturalistic study. *BMC Oral Health*. 2021;21(1):35. doi:10.1186/s12903-020-01379-x
46. Zhi QH, Lo ECM, Lin HC. Randomized clinical trial on effectiveness of silver diamine fluoride and glass ionomer in arresting dentine caries in preschool children. *J Dent*. 2012;40(11):962–967. doi:10.1016/j.jdent.2012.08.002
47. de França Lopes CMC, Schubert EW, Martins AS, et al. Randomized clinical trial of ART Class II restorations using two glass ionomer cements: One-year follow-up. *Pediatr Dent*. 2018;40(2):98–104. PMID:29663908.
48. Wafaie RA, Ibrahim Ali A, El-Negoly SAE, Mahmoud SH. Five-year randomized clinical trial to evaluate the clinical performance of high-viscosity glass ionomer restorative systems in small class II restorations. *J Esthet Restor Dent*. 2023;35(3):538–555. doi:10.1111/jerd.13000
49. Gopinath VK. Comparative evaluation of microleakage between bulk esthetic materials versus resin-modified glass ionomer to restore Class II cavities in primary molars. *J Indian Soc Pedod Prev Dent*. 2017;35(3):238–243. doi:10.4103/jispPd.Jisppd_17_17
50. Uzel İ, Aykut-Yetkiner A, Ersin N, Ertuğrul F, Atila E, Özcan M. Evaluation of glass-ionomer versus bulk-fill resin composite: A two-year randomized clinical study. *Materials (Basel)*. 2022;15(20):7271. doi:10.3390/ma15207271
51. Gurgan S, Kutuk ZB, Yalcin Cakir F, Ergin E. A randomized controlled 10 years follow up of a glass ionomer restorative material in class I and class II cavities. *J Dent*. 2020;94:103175. doi:10.1016/j.jdent.2019.07.013
52. Mahmood MA, Al-Hadithi HK, Hussein HM. Evaluation of dentin features in teeth after caries removal by three techniques (chemomechanical, mechanical with a smart bur, and air-abrasion): An in vitro study. *PeerJ*. 2024;12:e17717. doi:10.7717/peerj.17717

Original paper

Aromatherapy with lavender essential oil for the control of dental anxiety in patients undergoing dental surgery: A triple-blind randomized clinical trial

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Abstract

Background. Anxiety resulting from dental treatment often leads to avoidance of dental appointments and consequently has a negative impact on oral health. Non-pharmacological approaches have been identified as suitable alternatives for the management of dental anxiety. In this context, lavender oil has traditionally been used in aromatherapy (AT) due to its sedative, carminative, antidepressive, anti-inflammatory, and antimicrobial properties.

Objectives. The aim of the study was to perform a triple-blind randomized clinical trial to address the effectiveness of AT with lavender essential oil (ATL) on the dental anxiety of patients undergoing mandibular third molar surgery.

Material and methods. Participants scheduled to undergo mandibular third molar surgery were enrolled in the study and randomly assigned to either the ATL group or the control group. Dental anxiety was assessed using the preoperative Modified Dental Anxiety Scale (MDAS). Vital signs were evaluated before and after the intervention. Cortisol levels were measured by enzyme-linked immunosorbent assay (ELISA). Additionally, a visual analog scale (VAS) was used, and the requirements for anesthesia were determined.

Results. A comparison of the pre-operative MDAS scores between the ATL and control groups revealed no statistically significant differences ($p = 0.268$). The levels of respiratory rate (RR) ($p = 0.011$) and heart rate (HR) ($p = 0.021$) significantly decreased in the ATL group after the intervention. Also, the number of local anesthetic cartridges needed ($p = 0.030$) and cortisol levels ($p < 0.0001$) were significantly lower in the ATL group.

Conclusions. The evidence suggests that ATL could be helpful in the management of dental anxiety.

Keywords: dentistry, dental anxiety, complementary medicine

Highlights

- Aromatherapy with lavender essential oil significantly reduced salivary cortisol levels as well as post-intervention heart and respiratory rates compared with placebo.
- Although no statistically significant differences were observed in self-reported dental anxiety scores between groups, the aromatherapy group required significantly less local anesthetic during surgery, indirectly suggesting reduced anxiety or stress perception.
- Lavender aromatherapy was well tolerated, with no reported adverse effects or allergic reactions, supporting its safety and feasibility as a complementary approach for modulating acute stress responses in dental clinical practice.

Introduction

Herbal medicines encompass both unprocessed natural herbs and finished products that incorporate active ingredients derived from plants. The World Health Organization (WHO) has established that 80% of the global population relies on herbal medicine for their primary healthcare needs. Moreover, a quarter of pharmaceuticals used in developed countries include herbal compounds and their derivatives.[1] Herbal medicine, ozone and acupuncture have begun to be used as complementary therapies (CTs) in dentistry to relieve dental pain and pain associated with temporomandibular joint dysfunctions.[2] Also, CTs have been employed as coadjutants in the treatment of gingivitis, aphthous ulcers, and integrative head and neck cancer management.[3,4]

Aromatherapy (AT) is a CT that uses essential oils extracted from fresh plants for therapeutic purposes.[5] Aromatherapy consists of the application of essential oils to the skin through massage or inhalation of their vapors. Lavender oil is traditionally used in AT due to its sedative, carminative, antidepressive, anti-inflammatory, and antimicrobial effects.[6] The genus Lavandula encompasses 32 species, which fall into 4 main categories: *L. latifolia*; *L. angustifolia*; *L. stoechas*; and *L. intermedia.* These species share similar ethnobotanical properties and main chemical constituents, as evidenced by the sedative properties exhibited by both linalool and floral monoterpene.[7] Pharmacological studies in mice have associated the linalool odor with anxiolytic effects mediated by γ-aminobutyric acid (GABA)ergic transmission at the central nervous system (CNS).[8] Besides, linalool has demonstrated antidepressant and anti-stress effects mediated by an augmentation of serotonin and a diminution of cortisol levels in a rapid eye movement (REM) sleep-deprived model.[9] Likewise, linalool odor could modulate cortisol levels in humans subjected to experimental stress.[10]

Dental anxiety, defined as an irrational fear of undergoing dental treatment, is a prevalent condition that has been classified as a phobia.[11] The anxious behavior triggered by dental treatment leads patients to miss appointments and, therefore, has a negative impact on their oral health. The methods used to manage dental anxiety include psychophysiological therapy, premedication with anxiolytics, conscious sedation, and, on occasion, general anesthesia.[12] However, these approaches frequently have considerable adverse effects, making nonpharmacological methods potentially advisable options for treating dental anxiety.[13]

The present study aimed to perform a triple-blind randomized clinical trial to address the effectiveness of aromatherapy with lavender essential oil (ATL) on dental anxiety of patients undergoing third molar surgery.

Material and methods

Sample calculation and experimental group distribution

Power analysis was performed using G*Power software (v. 3.1.9.7; Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) to calculate the size of the study sample (input: two tails, effect size (d) = 0.5, α error probability = 0.05, power (1−β error probability) = 0.90, allocation ratio N2/N1 = 1).[14] The sample size of 90 individuals per group was determined. Patients undergoing mandibular third molar surgery were randomly allocated by the principal investigator (RTR) to one of 2 groups using random number generation with QuickCalcs in GraphPad Prism 9 software (GraphPad Software, La Jolla, USA). Another researcher enrolled the participants (CJSM), and a third researcher assigned the subjects to the interventions (EMVJ). The experimental group received ATL, while the control group received a placebo consisting of mineral oil (ATS) (Fig. 1).

Eligibility criteria

The included patients were apparently healthy participants of any sex, aged between 18 and 40 years, who required mandibular third molar surgery and agreed to participate in the study by signing the informed consent form. The exclusion criteria encompassed patients who did not meet the inclusion criteria or who had mental conditions, heart disease, upper respiratory tract infections, an allergy to lavender, or those undergoing drug

treatment for mental disorders. Additionally, patients who presented any complication of the surgical procedure and those who voluntarily withdrew from the study were excluded.

Intervention

Following previous reports, a medical patch containing 100 µL of lavender essential oil was placed in close proximity to the nose of each participant (avoiding skin contact) for 40 min before the dental surgery.[15,16] The essential oil of lavender (*L. angustifolia*), which had been marketed for clinical use, was obtained from PACALLI (Monterrey, México). The ATS group inhaled pure mineral oil in the same conditions.

Primary outcomes

Dental anxiety

Each participant completed the Modified Dental Anxiety Scale (MDAS) 45 min before the dental treatment. The MDAS is a questionnaire comprising 5 questions that are rated on a 5-point Likert scale. The total score is used to classify the level of anxiety, as follows: null anxiety; low anxiety; moderate anxiety; high anxiety; and severe anxiety. The precoded responses range from "null anxiety" (score 1) to "severe anxiety" (score 5).

Cortisol quantification

Saliva samples were collected 30 min after the administration of ATL or ATS. The samples were preserved at −80°C in polypropylene tubes before cortisol was quantified using the enzyme-linked immunosorbent assay (ELISA) (K008B; R&D Systems Inc., Minneapolis, USA) according to the manufacturer's instructions. The measurements were performed by a researcher who was blinded (LAF) regarding allocation to study groups.

Secondary outcomes

Vital signs

Heart rate (HR), respiratory rate (RR) and arterial pressure (AP) were assessed 5 min before ATL or ATS (denoted by the letter [b] next to the variable's abbreviation) and 30 min after the beginning of ATL or ATS (denoted by the letter [a] next to the variable's abbreviation) by a dental assistant who was blinded to study group allocation.

Pain

The surgeon, unaware of the study group allocation, used the visual analog scale (VAS) to assess pain levels. This continuous scale is represented by a horizontal line of 10 cm in length. At one extreme of the scale, no pain is represented by 0, while at the other extreme, unbearable pain is represented by 10. The score was determined by measuring the distance in centimeters using a ruler. The scale was assessed 5 min after the surgery.

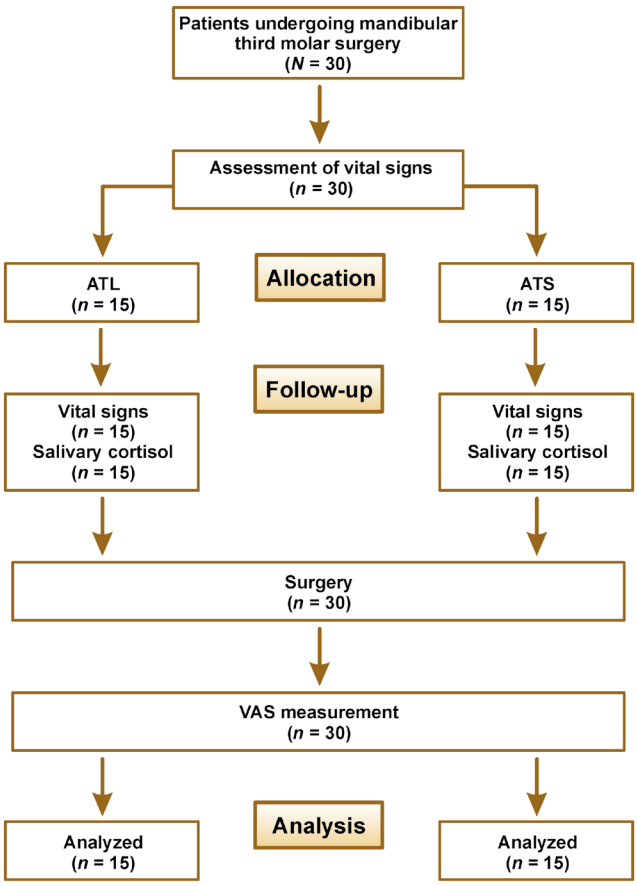

Fig. 1. Flowchart of the study

ATL – aromatherapy with lavender; ATS – sham aromatherapy; VAS – visual analog scale.

Anesthetic requirement

The dose of local anesthetic administered to each patient was recorded during the surgical procedure.

Statistical analysis

The data was analyzed using GraphPad Prism 9 software (GraphPad Software). The normality of the data was assessed by the Shapiro–Wilk test. The Student's *t*-test or Mann–Whitney *U* test was used to determine the mean or mean rank differences between the groups. The level of statistical significance was set at $p \leq 0.05$.

Results

The present clinical trial included 30 patients who underwent mandibular third molar surgery. Regarding

sex distribution, 53.3% of the participants were female and 46.7% were male. The mean age of the subjects was 26 years (Table 1). No participants withdrew from the study. Also, no cases of adverse effects or allergic reactions have been reported.

Table 1. Demographic characteristics of the participants

Group	Females, *n*	Males, *n*	Age [years] *M* (range)
ATS	8	7	26.6 (18–40)
ATL	8	7	26.4 (18–40)

ATL – aromatherapy with lavender; ATS – sham aromatherapy; *M* – mean.

The results of the normality test indicated that the data was normally distributed for MDAS, HR[b], RR[b], and cortisol level. On the other hand, the following variables did not follow a normal distribution: HR[a]; RR[a]; diastolic arterial pressure (DAP)[b]; DAP[a]; systolic arterial pressure (SAP)[a]; SAP[b]; VAS; and anesthetic requirement (AR).

Both groups displayed equal MDAS at baseline. Concerning the clinical sympathetic activity in the heart, HR in both groups showed similar results at baseline. However, after the intervention, HR exhibited lower values in the ATL group. On the other hand, for both groups, DAP and SAP demonstrated similar results at baseline and after the intervention. The respiratory rate in both groups presented similar results at baseline; however, after the intervention, lower RR values were noted in the ATL group. The cortisol level was significantly lower in the ATL group. Nevertheless, AR was significantly lower in the ATL group (Table 2).

Discussion

Dental fear is very common, and the coronavirus disease 2019 (COVID-19) pandemic caused an increase in dental anxiety levels among patients.[17–19] Several CTs have been studied for anxiety control, such as acupuncture, music therapy, massage, and AT.[20–25] However, conflicting results have been reported regarding the effectiveness of acupuncture, as it is an invasive therapy that involves the insertion of needles into muscle tissue. Furthermore, it would be difficult to administer acupuncture before the dental appointment, as it must be performed by a specialist.[26,27] On the other hand, music therapy has been shown to reduce salivary cortisol concentration, SAP, DAP, HR, body temperature, and stimulated salivary flow. However, it has been pointed out that the patient's musical preferences may influence the results, and that music may function more as a distraction than as a therapy in itself if it is not delivered directly to the patient through headphones. Additional concerns have been made regarding the hygiene of the headphones or the potential financial implications for the patient.[28,29] Massage, like acupuncture, necessitates the expertise of a specialist and a suitable environment for its administration prior to the dental appointment, which makes this option impractical.[30]

Essential oils extracted from aromatic herbal sources have sedative and stress-relieving properties.[31] The evidence for these properties has been obtained through in vivo studies and clinical trials in humans, which demonstrated the physiological effects of odorant inhalation.[8] In some clinical studies, ATL was used to alleviate dental anxiety and has been considered effective. However, the majority of studies used questionnaires to evaluate the degree of anxiety and to measure vital signs in both children and adults undergoing dental procedures.[32–36]

The mechanisms of AT are predicated on the activation of the sense of smell, which has significant effects on the body. The odorant inhalation acts on the olfactory pathway, extending from the nasal cavity to the olfactory bulb. The pathway sends impulses to the limbic system, which modulates the CNS and induces nerve-calming and mood-enhancing effects. Linalool is a main component of essential oils that are used as anxiolytic agents. They exhibit a dose-dependent impact on the CNS and interact with monoamine oxidase A (MAO-A), the serotonin transporter (SERT), GABA, and N-methyl-D-aspartate (NMDA) receptors.[37] Also, linalool reduces the levels of catecholamines within the CNS and suppresses the activity of sympathetic nerves that innervate the adipose tissues and adrenal gland, modulating sympathetic nerve activity and blood pressure.[38–42] In the current study,

Table 2. Comparison of clinical outcomes between the study groups

Group	MDAS[##]	HR[b##] [beats/min]	HR[a#] [beats/min]	RR[b##] [breaths/min]	RR[a#] [breaths/min]	DAP[b#] [mmHg]	DAP[a#] [mmHg]	SAP[b#] [mmHg]	SAP[a#] [mmHg]	VAS[#]	AR[#] [mL]	Cortisol[##] [nmol/L]
ATS	12.40 ±2.50	70.67 ±2.16	69.33 ±4.64	20.20 ±2.15	19.47 ±2.03	65.33 ±8.34	68.33 ±6.46	103.30 ±9.00	106.20 ±6.28	1.13 ±2.07	4.68 ±1.64	17.55 ±4.66
ATL	11.87 ±1.85	73.33 ±2.95	64.27 ±8.88	20.60 ±2.50	17.13 ±2.30	70.00 ±8.45	66.67 ±6.46	105.90 ±8.10	101.30 ±5.50	0.20 ±0.41	3.48 ±1.07	5.90 ±1.95
p-value	0.268	0.255	0.021*	0.247	0.011*	0.107	0.676	0.365	0.053	0.274	0.030*	<0.0001*

Data presented as *M* ± standard deviation (*SD*); * statistically significant ($p \leq 0.05$); [#] Mann–Whitney test; [##] Student's *t*-test; [b] before ATS or ATL; [a] after ATS or ATL; MDAS – Modified Dental Anxiety Scale; HR – heart rate; RR – respiratory rate; DAP – diastolic arterial pressure; SAP – systolic arterial pressure; VAS – visual analog scale; AR – anesthetic requirement.

patients receiving ATL showed lower HR and RR levels compared to the ATS group. However, the AP values did not exhibit statistical differences between the groups. These results can be attributed to the normality in the parameters of AP or the dose-dependent effects of linalool.

Linalool has a wide range of physiological effects, including pain management.[43,44] For instance, it has been reported to diminish orofacial pain by activating inhibitory neurotransmission in the substantia gelatinosa of the trigeminal subnucleus caudalis.[45] However, in the present study, patients receiving ATL or ATS did not demonstrate significant differences in pain evaluation. The observed outcomes may be related to the limited duration of follow-up, the measurement methodology, or the dose-dependent effects of linalool.

In addition, it has been reported that the administration of AT with aromatic essential oils containing linalool reduced cortisol levels in the saliva of healthy volunteers under conditions of standardized stress.[10,46]

In the present study, patients receiving ATL presented lower cortisol levels compared to the ATS group, which could be related to a low level of anxiety in the ATL group. Likewise, only 1 clinical study with the ATL group measured cortisol levels in children after the administration of local anesthetics. The study noted a lower amount of cortisol in the intervention group compared to the control group.[47]

The United States Food and Drug Administration (FDA) has classified lavender as a safe substance.[48] Products containing lavender are available on the market for the treatment of insomnia and anxiety. However, these products are offered as dietary supplements that do not require extensive pre-marketing approval from the FDA. Nonetheless, the administration of ATL in the waiting room of a dental office can be an easy and safe technique to reduce anxiety in patients.

Limitations

Numerous studies have noted that learning experiences of an aversive or traumatic nature are associated with stress-related problems and specific phobias.[49] Also, dental anxiety and fear have been related to the dental traumatic story, cognition and perception.[50] Therefore, one limitation of this study is the lack of analysis of cortisol levels, the association of MDAS with traumatic dental experiences, and patients' educational level. Another limitation may be related to the environmental conditions of the study. The waiting room of a dental teaching clinic was consistently used at the same time of day, and the intervention was conducted over a short period (1 month), during which environmental conditions showed little variation and ambient temperature remained similar. However, in the present study, the volatile fraction of the compounds in lavender essential oil was not determined. Additionally, the target sample size of 90 individuals per group was not achieved. Nonetheless, a sample calculation proposed by Whitehead et al., based on a non-central t-distribution approach, indicated that 15 participants per group were sufficient for obtaining preliminary results.[51]

Conclusions

Aromatherapy with lavender may reduce HR, RT and cortisol levels in patients undergoing mandibular third molar surgery, presumably by alleviating anxiety. Future trials with adequate sample sizes that examine correlations among variables and compare ATL with other standard anxiety treatments may help generate more robust conclusions.

Trial registration

This parallel, triple-blind randomized clinical trial was registered at ClinicalTrials.gov in February 2020 (ID No. NCT04285385; https://clinicaltrials.gov/study/NCT04285385).

Ethics approval and consent to participate

The study was approved by the Bioethics Committee for Research, Faculty of Dentistry, Benito Juárez Autonomous University of Oaxaca (UABJO) (Oaxaca de Juárez, Mexico) in January 2020 (approval No. 19PEC001FO). All subjects provided written informed consent, and the intervention was conducted in accordance with the Declaration of Helsinki, including the current revisions, Good Clinical Practice guidelines, and the University Manual of Ethics Operations in Research (Benito Juárez Autonomous University of Oaxaca (UABJO)).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Kumar G, Jalaluddin M, Rout P, Mohanty R, Dileep CL. Emerging trends of herbal care in dentistry. *J Clin Diag Res*. 2013;7(8):1827–1829. doi:10.7860/JCDR/2013/6339.3282
2. Benli M, Olson J, Huck O, Özcan M. A novel treatment modality for myogenous temporomandibular disorders using aromatherapy massage with lavender oil: A randomized controlled clinical trial. *Cranio*. 2020:41(1):48–58. doi:10.1080/08869634.2020.1819067
3. Jimson S, Malathi L, Devi GN, Sankari SL. Aromatherapy in dentistry – a review. *Biomed Pharmacol J*. 2016;9(2):827–828. doi:10.13005/bpj/1010
4. Bautista-Hernández MA, Castillo-Real LM, Castro-Gutiérrez MEM, Gijón-Soriano AL, Argueta-Figueroa L. Complementary therapies in the integrative management of head and neck cancer: A scoping review [in Spanish]. *Rev Int Acupunt*. 2021;15(3):100151. doi:10.1016/j.acu.2021.05.001
5. Halcón LL. Aromatherapy: Therapeutic applications of plant essential oils. *Minn Med*. 2002;85(11):42–46. PMID:12498066.
6. Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, Anwar F. Essential oils used in aromatherapy: A systemic review. *Asian Pac J Trop Biomed*. 2015;5(8):601–611. doi:10.1016/j.apjtb.2015.05.007
7. Cavanagh HMA, Wilkinson JM. Biological activities of lavender essential oil. *Phytother Res*. 2002;16(4):301–308. doi:10.1002/ptr.1103
8. Harada H, Kashiwadani H, Kanmura Y, Kuwaki T. Linalool odor-induced anxiolytic effects in mice. *Front Behav Neurosci*. 2018;12:241. doi:10.3389/fnbeh.2018.00241
9. Lee BK, Jung AN, Jung YS. Linalool ameliorates memory loss and behavioral impairment induced by REM-sleep deprivation through the serotonergic pathway. *Biomol Ther (Seoul)*. 2018;26(4):368–373. doi:10.4062/biomolther.2018.081
10. Höferl M, Krist S, Buchbauer G. Chirality influences the effects of linalool on physiological parameters of stress. *Planta Med*. 2006;72(13):1188–1192. doi:10.1055/s-2006-947202
11. Oosterink FMD, de Jongh A, Hoogstraten J. Prevalence of dental fear and phobia relative to other fear and phobia subtypes. *Eur J Oral Sci*. 2009;117(2):135–143. doi:10.1111/j.1600-0722.2008.00602.x
12. Fábián TK, Fejérdy P, Hermann P, Fábián G. Chapter 60 – Dental Stress. In: Fink G, ed. *Stress: Concepts, Cognition, Emotion, and Behavior*. San Diego, CA: Academic Press; 2016:473–477. doi:10.1016/B978-0-12-800951-2.00066-2
13. Becker DE. Pharmacodynamic considerations for moderate and deep sedation. *Anesth Prog*. 2012;59(1):28–42. doi:10.2344/0003-3006-59.1.28
14. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39(2):175–191. doi:10.3758/bf03193146
15. Asgari MR, Vafaei-Moghadam A, Babamohamadi H, Ghorbani R, Esmaeili R. Comparing acupressure with aromatherapy using *Citrus aurantium* in terms of their effectiveness in sleep quality in patients undergoing percutaneous coronary interventions: A randomized clinical trial. *Complement Ther Clin Pract*. 2020;38:101066. doi:10.1016/j.ctcp.2019.101066
16. Kim JT, Wajda M, Cuff G, et al. Evaluation of aromatherapy in treating postoperative pain: Pilot study. *Pain Pract*. 2006;6(4):273–277. doi:10.1111/j.1533-2500.2006.00095.x
17. Kaya N, Bayındır F. Evaluation of the relationship between the geriatric anxiety and COVID-19 anxiety and fear levels in geriatric dental patients during the COVID-19 pandemic. *Dent Med Probl*. 2023;60(1):5–11. doi:10.17219/dmp/157345
18. Berberoğlu B, Koç N, Boyacioglu H, et al. Assessment of dental anxiety levels among dental emergency patients during the COVID-19 pandemic through the Modified Dental Anxiety Scale. *Dent Med Probl*. 2021;58(4):425–432. doi:10.17219/dmp/139042
19. Łazarz-Półkoszek MJ, Orczykowska M, Gala A, Pihut M. Impact of the COVID-19 pandemic on patients' anxiety levels related to dental appointments in Poland. *Dent Med Probl*. 2023;60(3):367–373. doi:10.17219/dmp/163476
20. del Río Medina S, Martínez-González MC, Montero Navarro S, Sánchez-Más J, Caparrós Granados M. Acupuncture treatment of pre-examination anxiety in university students [in Spanish]. *Rev Int Acupunt*. 2019;13(1):2–11. doi:10.1016/j.acu.2019.03.001
21. Baesso Cavalca AM, Gomes C, Nogueira DA, Rossi Junior WC, dos Santos de Almeida M. The effect of acupuncture on exam anxiety in medical students: A randomized crossover study. *Rev Int Acupunt*. 2019;13(2):43–48. doi:10.1016/j.acu.2019.09.001
22. Abuye NO, Sánchez-Pérez I. Effectiveness of acupuncture and auriculotherapy to reduce the level of depression, anxiety and stress in emergency health personnel during the COVID-19 pandemic [in Spanish]. *Rev Int Acupunt*. 2021;15(2):43–50. doi:10.1016/j.acu.2021.04.001
23. Simkin DR, Swick S, Taneja KS, Ranjbar N. Complementary and integrative medicine for anxiety in children, adolescents, and young adults. *Child Adolesc Psychiatr Clin N Am*. 2023;32(2):193–216. doi:10.1016/j.chc.2022.08.006
24. Ebrahimi A, Eslami J, Darvishi I, Momeni K, Akbarzadeh M. Investigation of the role of complementary medicine on anxiety of patients before and after surgery: A review study. *Holist Nurs Pract*. 2020;34(6):365–379. doi:10.1097/hnp.0000000000000414
25. Umbrello M, Sorrenti T, Mistraletti G, Formenti P, Chiumello D, Terzoni S. Music therapy reduces stress and anxiety in critically ill patients: A systematic review of randomized clinical trials. *Minerva Anestesiol*. 2019;85(8):886–898. doi:10.23736/s0375-9393.19.13526-2
26. Allan FK, Peckham E, Liu J, et al. Acupuncture for anxiety in dental patients: Systematic review and meta-analysis. *Eur J Integr Med*. 2018;20:22–35. doi:10.1016/j.eujim.2018.03.008
27. Armond ACV, Glória JCR, Dos Santos CRR, Galo R, Falci SGM. Acupuncture on anxiety and inflammatory events following surgery of mandibular third molars: A split-mouth, randomized, triple-blind clinical trial. *Int J Oral Maxillofac Surg*. 2019;48(2):274–281. doi:10.1016/j.ijom.2018.07.016
28. Mejía-Rubalcava C, Alanís-Tavira J, Mendieta-Zerón H, Sánchez-Pérez L. Changes induced by music therapy to physiologic parameters in patients with dental anxiety. *Complement Ther Clin Pract*. 2015;21(4):282–286. doi:10.1016/j.ctcp.2015.10.005
29. Bradt J, Teague A. Music interventions for dental anxiety. *Oral Dis*. 2018;24(3):300–306. doi:10.1111/odi.12615
30. Liu T. Role of acupuncturists in acupuncture treatment. *Evid Based Complement Alternat Med*. 2007;4(1):3–6. doi:10.1093/ecam/nel061
31. Sattayakhom A, Wichit S, Koomhin P. The effects of essential oils on the nervous system: A scoping review. *Molecules*. 2023;28(9):3771. doi:10.3390/molecules28093771
32. Alkanan SAM, Alhaweri HS, Khalifa GA, Ata SMS. Dental pain perception and emotional changes: On the relationship between dental anxiety and olfaction. *BMC Oral Health*. 2023;23(1):175. doi:10.1186/s12903-023-02864-9
33. Arslan I, Aydinoglu S, Karan NB. Can lavender oil inhalation help to overcome dental anxiety and pain in children? A randomized clinical trial. *Eur J Pediatr*. 2020;179(6):985–992. doi:10.1007/s00431-020-03595-7
34. Janthasila N, Keeratisiroj O. Music therapy and aromatherapy on dental anxiety and fear: A randomized controlled trial. *J Dent Sci*. 2023;18(1):203–210. doi:10.1016/j.jds.2022.06.008
35. Kritsidima M, Newton T, Asimakopoulou K. The effects of lavender scent on dental patient anxiety levels: A cluster randomised-controlled trial. *Community Dent Oral Epidemiol*. 2010;38(1):83–87. doi:10.1111/j.1600-0528.2009.00511.x
36. Nirmala K, Kamatham R. Effect of aromatherapy on dental anxiety and pain in children undergoing local anesthetic administrations: A randomized clinical trial. *J Caring Sci*. 2021;10(3):111–120. doi:10.34172/jcs.2021.026
37. López V, Nielsen B, Solas M, Ramírez MJ, Jäger AK. Exploring pharmacological mechanisms of lavender (*Lavandula angustifolia*) essential oil on central nervous system targets. *Front Pharmacol*. 2017;8:280. doi:10.3389/fphar.2017.00280
38. Cheng BH, Sheen LY, Chang ST. Evaluation of anxiolytic potency of essential oil and S-(+)-linalool from *Cinnamomum osmophloeum* ct. linalool leaves in mice. *J Tradit Complement Med*. 2015;5(1):27–34. doi:10.1016/j.jtcme.2014.10.007
39. Nagai K, Niijima A, Horii Y, Shen J, Tanida M. Olfactory stimulatory with grapefruit and lavender oils change autonomic nerve activity and physiological function. *Auton Neurosci*. 2014;185:29–35. doi:10.1016/j.autneu.2014.06.005

40. Tanida M, Niijima A, Shen J, Nakamura T, Nagai K. Olfactory stimulation with scent of lavender oil affects autonomic neurotransmission and blood pressure in rats. *Neurosci Lett*. 2006;398(1–2):155–160. doi:10.1016/j.neulet.2005.12.076
41. Yamada K, Mimaki Y, Sashida Y. Effects of inhaling the vapor of *Lavandula burnatii* super-derived essential oil and linalool on plasma adrenocorticotropic hormone (ACTH), catecholamine and gonadotropin levels in experimental menopausal female rats. *Biol Pharm Bull*. 2005;28(2):378–379. doi:10.1248/bpb.28.378
42. Arriaga-Pizano L, Gómez-Jiménez DC, Flores-Mejía LA, et al. Low back pain in athletes can be controlled with acupuncture by a catecholaminergic pathway: Clinical trial. *Acupunct Med*. 2020;38(6):388–395. doi:10.1177/0964528420912251
43. Donatello NN, Emer AA, Salm DC, et al. *Lavandula angustifolia* essential oil inhalation reduces mechanical hyperalgesia in a model of inflammatory and neuropathic pain: The involvement of opioid and cannabinoid receptors. *J Neuroimmunol*. 2020;340:577145. doi:10.1016/j.jneuroim.2020.577145
44. Raisi Dehkordi Z, Hosseini Baharanchi FS, Bekhradi R. Effect of lavender inhalation on the symptoms of primary dysmenorrhea and the amount of menstrual bleeding: A randomized clinical trial. *Complement Ther Med*. 2014;22(2):212–219. doi:10.1016/j.ctim.2013.12.011
45. Phuong TNT, Jang SH, Rijal S, et al. GABA- and glycine-mimetic responses of linalool on the substantia gelatinosa of the trigeminal subnucleus caudalis in juvenile mice: Pain management through linalool-mediated inhibitory neurotransmission. *Am J Chin Med*. 2021;49(6):1437–1448. doi:10.1142/s0192415x21500671
46. Chaiyasut C, Sivamaruthi BS, Wongwan J, et al. Effects of *Litsea cubeba* (Lour.) persoon essential oil aromatherapy on mood states and salivary cortisol levels in healthy volunteers. *Evid Based Complement Alternat Med*. 2020;2020:4389239. doi:10.1155/2020/4389239
47. Ghaderi F, Solhjou N. The effects of lavender aromatherapy on stress and pain perception in children during dental treatment: A randomized clinical trial. *Complement Ther Clin Pract*. 2020;40:101182. doi:10.1016/j.ctcp.2020.101182
48. Food and Drug Administration, Department of Health and Human Services. 21 CFR 182.20. Essential oils, oleoresins (solvent-free), and natural extractives (including distillates). https://www.ecfr.gov/current/title-21/chapter-I/subchapter-B/part-182/subpart-A/section-182.20. Accessed January 4, 2022.
49. Coelho CM, Purkis H. The origins of specific phobias: Influential theories and current perspectives. *Rev Gen Psychol*. 2009;13(4):335–348. doi:10.1037/a0017759
50. Armfield JM. Towards a better understanding of dental anxiety and fear: Cognitions vs. experiences. *Eur J Oral Sci*. 2010;118(3):259–264. doi:10.1111/j.1600-0722.2010.00740.x
51. Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. *Stat Methods Med Res*. 2016;25(3):1057–1073. doi:10.1177/0962280215588241

Original paper

Comparative efficacy of a local drug delivery system using Blue®M gel and Gengigel® as an adjunct to scaling and root planing in chronic periodontitis patients

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D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. Periodontal disease is a condition that impacts the tissues surrounding the teeth, sparked by the build-up of bacteria leading to inflammation. These bacteria form biofilms, shielding themselves from antimicrobial treatment. Typically, mechanical cleaning disrupts these biofilms and lowers bacterial levels. However, in some cases, it might not fully address the disease due to intricate root structures and irregular lesion shapes. Local drug delivery directly to the affected area serves as a reservoir for drugs, potentially altering harmful bacteria and aiding in the healing process.

Objectives. The aim of the study was to evaluate and compare the effectiveness of various local drug delivery systems, specifically Blue®M gel along with scaling and root planing (SRP), Gengigel® with SRP, and SRP alone, in managing patients with chronic periodontitis.

Material and methods. The study population comprised 36 patients of both sexes in the age group of 35–60 years, with supra-bony pockets of 4–6 mm in any quadrant. The participants were randomly divided into 3 groups according to the treatment provided: group A – SRP alone; group B – SRP with BlueM gel; and group C – SRP with Gengigel. The gels were applied into the pockets. The follow-up period was 6 months.

Results. In a study involving 36 patients, predominantly males, with an average age of 42.03 years, Gengigel demonstrated a slightly greater reduction in the probing pocket depth (PPD) (–0.16 ±0.04 mm), the oral hygiene index (OHI) (–0.45 ±0.81) and the plaque index (PI) (–0.51 ±0.33) as compared to the other 2 groups. BlueM gel exhibited a superior reduction in the clinical attachment level (CAL) when compared to the control and Gengigel groups (–0.35 ±0.31 mm).

Conclusions. Both BlueM Gel and Gengigel have proven to be dependable choices or adjuncts to SRP. However, additional studies are needed to further explore their effectiveness.

Keywords: periodontitis, local drug delivery, scaling and root planing, Blue®M gel, Gengigel®

Highlights

- The adjunctive use of Blue®M gel and Gengigel® with scaling and root planing (SRP) produced significantly greater improvement in periodontal parameters after 6 months as compared to SRP alone.
- Gengigel® showed superior reductions in the probing pocket depth (PPD), the oral hygiene index (OHI) and the plaque index (PI).
- Blue®M gel demonstrated greater improvement in the clinical attachment level (CAL) than both Gengigel® and SRP alone.
- Both local drug delivery systems were safe and effective adjuncts to non-surgical periodontal therapy, supporting their clinical use in managing stage II grade B chronic periodontitis.

Introduction

Periodontitis is a persistent inflammatory condition that undermines the structural integrity of the tissues supporting the teeth. These tissues include the gingiva, the periodontal ligament and the alveolar bone, collectively called the periodontium.[1] Chronic inflammation associated with periodontal disease compromises the integrity of the periodontal tissues, which is a crucial barrier against microbial invasion. This loss of integrity has adverse effects on the underlying periodontal tissues.[2] The primary cause of periodontal disease is plaque on the surface of the tooth, causing the inflammation of marginal tissues; a condition known as gingivitis, which, if left untreated, may progress to periodontitis.[3,4] Surface root debridement, accepted as the most effective approach for treating periodontitis, involves removing the biofilm and bacterial products on the surfaces of the afflicted roots surfaces.[5,6] Despite its efficacy, scaling and root planing (SRP) might be technically challenging and inconsistent in eradicating all periodontal bacteria or reducing inflammation in periodontitis.[2] Therefore, systemic and local antimicrobial therapies are often used as adjuncts to mechanical surface root debridement to enhance clinical outcomes.

Local drug therapy is frequently employed in managing periodontal disease, offering site-specific delivery of medications precisely where needed for an adequate duration. This approach ensures effective treatment without subjecting the entire body to therapeutic agents.[7]

Oxygen is a vital nutrient in cellular metabolism, particularly in energy generation. It is closely implicated in several physiological processes, including oxidative bacterial clearance, tissue re-epithelialization, angiogenesis, and collagen formation.[8] Under controlled concentrations, reactive oxygen species (ROS) can transform into free radicals, promoting the growth of fibroblasts and epithelial cells. However, elevated concentrations of these free radicals may lead to tissue injury.[9] Dr. Peter Blijdorp from the Netherlands[10] developed a product named Blue®M based on active oxygen, comprising sodium perborate, glucose oxidase enzyme derived from honey, xylitol, and lactoferrin.[11] These components are thoughtfully combined to harness the benefits of active oxygen while ensuring a balanced and controlled application, potentially supporting wound healing and tissue regeneration. Research by Koul et al.,[9] Deliberador et al.[11] and Asha et al.[12] suggests that BlueM gel could be considered a viable alternative or adjunct to SRP in managing periodontal disease.

The antibacterial and anti-inflammatory properties of hyaluronic acid (HA) have positioned it as a valuable local chemotherapeutic agent in treating periodontal disease.[13] Beyond its antibacterial efficacy, HA is recognized for its antifungal effects, contributing to its versatility in combating microbial infections. Additionally, it exhibits angiogenic and osteoinductive properties, further enhancing the healing process within the periodontal tissues.[14] Gengigel®, which contains HA, is crucial in promoting the formation of blood vessels, the granulation tissue and osteoblasts.[15] The role of HA is pivotal in modulating the fibrin network, the granulation tissue and the provisional matrix, which are critical to periodontal regeneration. A collective body of research, including studies by Mohammad et al.,[5] Suchetha et al.,[16] Sahayata et al.,[17] Kalra et al.,[18] and Sandhu et al.,[19] has evidenced the therapeutic benefits of using HA. Notably, Rodríguez-Aranda et al.'s systematic review of 9 studies corroborated the efficacy of HA in improving clinical periodontal indices – the clinical attachment level (CAL), the probing pocket depth (PPD) and bleeding on probing (BoP) – both as standalone treatment and as a complement to surgical interventions like flap debridement, bone grafting, and the use of growth factors.[14]

Our study ventured beyond the individual assessments of BlueM gel and Gengigel, engaging in a direct comparison between the two. We evaluated their impact on the key periodontal parameters – PPD, CAL, the oral hygiene index (OHI), and the plaque index (PI). This study is the first to assess the efficacy of BlueM gel and Gengigel when each is combined with SRP, as well as SRP alone. The study aimed to provide insights into the potential benefits of these interventions in enhancing the outcomes of periodontal therapy.

Material and methods

Preliminary plan and ethics statement

This study was a prospective, randomized controlled trial (RCT), conducted at MNR Dental College and Hospital, Sangareddy, India, with the approval of the institutional review board (IRB) (MNR EC-BHR-07/23). The participants were fully briefed about the study and consented in writing. Employing randomization minimized variability and bias, ensuring standardized test site selection across the groups from the outset and enhancing the reliability of the results.

Inclusion and exclusion criteria

Patients were selected from the ambulatory care unit of the Department of Periodontology at MNR Dental College and Hospital in accordance with the guidelines of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.[20] The inclusion criteria comprised individuals aged 35–60 yars of both genders, diagnosed with chronic periodontitis (stage II grade B periodontitis). Eligible patients presented with at least one site with PPD of 4–6 mm and CAL of 4–6 mm, along with horizontal bone loss, necessitating non-surgical treatment. The study excluded individuals with systemic diseases, those on antibiotics or anti-inflammatory medications, smokers, tobacco or alcohol users, immunocompromised persons, pregnant or lactating women, non-compliant or physically challenged individuals, and those unable to maintain good oral hygiene or attend follow-ups.

Sample size calculation

Sample size calculation was based on a study by Manjunatha et al.[21] A one-way analysis of variance (ANOVA) with fixed effects was employed to assess the data. An a priori analysis was conducted to calculate the necessary sample size, using an effect size of 0.55, an alpha error probability of 0.05, and a power of 0.80 across 3 groups. This analysis yielded a required total sample size of 36, divided evenly into 12 participants per group. The output indicated a non-centrality parameter (λ) of 10.89, a critical F-value of 3.285, numerator degrees of freedom (*dfn*) of 2, denominator degrees of freedom (*dfd*) of 33, and an actual power of 0.813.

Estimation of clinical parameters

After the initial screening, all patients underwent supragingival full-mouth SRP. A total of 36 patients meeting the inclusion and exclusion criteria were chosen. The PI,[22] OHI,[23] PPD (measured with the UNC-15 periodontal probe), and CAL values were recorded at baseline and 6 months post-treatment.

Randomization

The allocation of 36 sites from 36 patients was randomized using the Research Randomizer software, v. 2.0 (https://www.randomizer.org).

Gel composition

A qualified clinician administered the allocated treatment plan to the selected sites for each group. The control group received treatment with SRP using UNC-15. In contrast, the experimental group sites were treated with SRP and either BlueM gel or Gengigel.

BlueM gel (Bluemcare India; New Delhi, India) composition: aqua; alcohol; glycerin; silica; sodium saccharin; sodium perborate; citric acid; PEG-32; sodium gluconate; lactoferrin; xanthan gum; cellulose gum.

Gengigel (Ricerfarma, Milan, Italy) composition: aqua; xylitol; cellulose gum; alcohol; PEG-40 hydrogenated castor oil; poly(vinyl) alcohol (PVA); polycarbophil; dichlorobenzyl alcohol; aroma (CITROMINT 1/074600); sodium hydroxide; Acid Blue 9 (CI 42090).

Intracrevicular administration of BlueM gel and Gengigel

The study utilized BlueM gel and Gengigel as the study materials. The dental arches were divided into 4 quadrants, each receiving a different treatment modality: group A – SRP alone; group B – SRP with BlueM gel; and group C – SRP with Gengigel. All patients in the 3 groups underwent a pre-surgical protocol that included the collection of pretreatment records, such as detailed medical and dental histories, thorough clinical examinations, clinical photographs, and essential laboratory investigations. The gels were loaded into a syringe and placed intra-sulcularly in the selected area. The site was then covered with a periodontal Coe-Pack, and the patients were instructed to refrain from eating coarse foods, drinking, brushing, or rinsing the treated area for 24 h following application (Fig. 1 and 2).

Statistical analysis

All statistical calculations were performed using IBM SPSS Statistics for Windows, v. 21.0 (IBM Corp., Armonk, USA). All values were presented as mean and standard deviation ($M \pm SD$). ANOVA and paired t tests were used for the comparative analysis of the data.

Results

Among the 36 patients, 19 (52.78%) were male and 17 (47.22%) were female, with the average age of 42.03 ±8.70 years. The PPD depth measurement was conducted at the

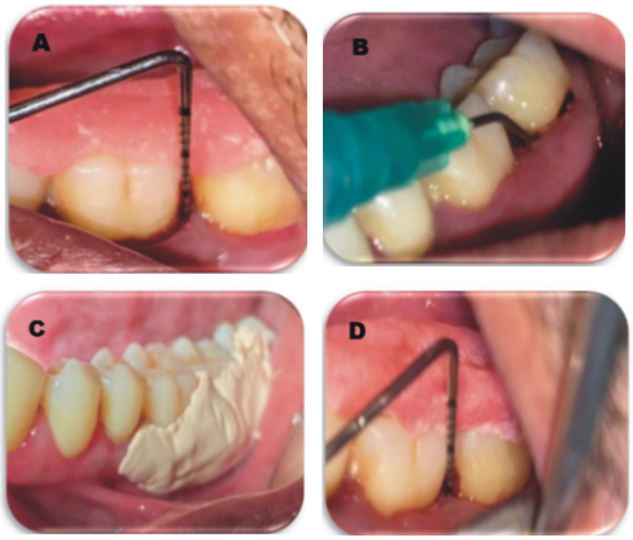

Fig. 1. Administration of BlueM gel, and the pre- and post-operative measurement of the probing pocket depth (PPD)

A – pre-operative measurement of PPD; B – insertion of BlueM gel; C – application of a periodontal Coe-Pack dressing; D – post-operative measurement of PPD.

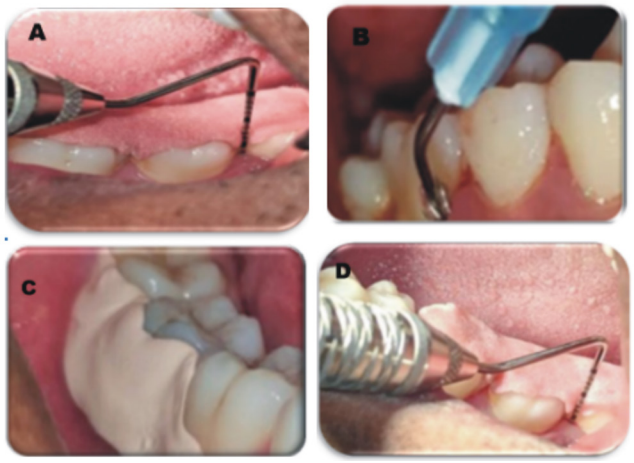

Fig. 2. Administration of Gengigel, and the pre- and post-operative measurement of the probing pocket depth (PPD)

A – pre-operative measurement of PPD; B – insertion of Gengigel; C – application of a periodontal Coe-Pack dressing; D – post-operative measurement of PPD.

drug delivery site, with the initial value recorded during the baseline visit, on the day of drug delivery. Subsequently, another PPD measurement was taken and recorded during the reassessment visit (Fig. 1 and 2). A paired *t* test with a 95% confidence interval (*CI*) was performed to compare the mean difference in PPD between the BlueM gel and the Gengigel groups. The results indicated a statistically significant *p*-value for the mean difference between baseline and the follow-up at 6 months with regard to PPD (Table 1), CAL (Table 2), OHI (Table 3), and PI (Table 4).

All 3 groups showed a significant reduction in PPD at 6 months post-treatment, with group C (Gengigel) demonstrating a slightly greater reduction than the other 2 groups. The mean reduction in the Gengigel group was

Table 1. Mean difference in the probing pocket depth (PPD) between the measurements performed at baseline and at 6 months post-treatment

Group	Mean difference in PPD [mm] between baseline and the follow-up at 6 months	*t*-value	*p*-value
Group A	−0.09 ±0.03	−6.17	<0.001*
Group B	−0.11 ±0.19	−2.78	0.019*
Group C	−0.16 ±0.04	−9.67	<0.001*

Data presented as mean ± standard deviation (*M* ±*SD*).
Groups: A – control (scaling and root planing (SRP) alone); B – SRP with BlueM gel; C – SRP with Gengigel. * statistically significant.

Table 2. Mean difference in the clinical attachment level (CAL) between the measurements performed at baseline and at 6 months post-treatment

Group	Mean difference in CAL [mm] between baseline and the follow-up at 6 months	*t*-value	*p*-value
Group A	−0.15 ±0.13	−4.78	<0.001*
Group B	−0.35 ±0.31	−7.22	<0.001*
Group C	−0.21 ±0.19	−5.50	<0.001*

Data presented as *M* ±*SD*.
Groups: A – control (scaling and root planing (SRP) alone); B – SRP with BlueM gel; C – SRP with Gengigel. * statistically significant.

Table 3. Mean difference in the oral hygiene index (OHI) between the measurements performed at baseline and at 6 months post-treatment

Group	Mean differencein OHI between baseline and the follow-up at 6 months	*t*-value	*p*-value
Group A	−0.18 ±0.06	−8.85	<0.001*
Group B	−0.38 ±0.26	−8.69	<0.001*
Group C	−0.45 ±0.81	−5.74	<0.001*

Data presented as *M* ±*SD*.
Groups: A – control (scaling and root planing (SRP) alone); B – SRP with BlueM gel; C – SRP with Gengigel. * statistically significant.

Table 4. Mean difference in the plaque index (PI) between the measurements performed at baseline and at 6 months post-treatment

Group	Mean difference in PI between baseline and the follow-up at 6 months	*t*-value	*p*-value
Group A	−0.24 ±0.19	−6.38	<0.001*
Group B	−0.27 ±0.18	−7.39	<0.001*
Group C	−0.51 ±0.33	−10.18	<0.001*

Data presented as *M* ±*SD*.
Groups: A – control (scaling and root planing (SRP) alone); B – SRP with BlueM gel; C – SRP with Gengigel. * statistically significant.

−0.16 ±0.04 mm, followed by −0.11 ±0.19 mm in the BlueM gel group, and −0.09 ±0.03 in controls.

After the 6-month period, all 3 groups demonstrated a noteworthy reduction in CAL. Group B (BlueM gel) exhibited a slightly superior reduction than the other 2 groups. Specifically, the mean reduction in group B was −0.35 ±0.31 mm, group C recorded −0.21 ±0.19 mm, and group A displayed −0.15 ±0.13 mm.

At the reassessment visit, all 3 groups exhibited a substantial reduction in OHI. The Gengigel group (C) notably demonstrated a slightly superior reduction than the BlueM gel and control groups. The mean reduction in group C was −0.45 ±0.81, group B showed −0.38 ±0.26, and group A exhibited −0.18 ±0.06.

Upon completing the 6-month period, a substantial reduction in PI was observed in all 3 groups. Group C (Gengigel) showed a slightly superior reduction than the other 2 groups. The mean reduction in group C was −0.51 ±0.33, while group B showed −0.27 ±0.18, and group A exhibited −0.24 ±0.19.

Discussion

The foundation of periodontal therapy lies in non-surgical mechanical treatment, which represents the primary and recommended approach for controlling periodontal infections. Additionally, the local subgingival application of chemotherapeutic agents can augment the clinical and microbiological benefits of mechanical debridement. Oral hygiene products supplemented with chemotherapeutic agents, offering diverse antimicrobial actions, have been shown to be effective adjuncts when used alongside mechanical plaque control.[24]

In the present study, all 3 treatment modalities resulted in a significant reduction in the clinical parameters. Clinical studies have demonstrated the osteoinductive properties of hyaluronan, as it stimulates osteoprogenitor cells within the defect. This stimulation leads to successive differentiation into osteoblasts and the formation of new bone.[5,25] A systematic review by Rodríguez et al., which included 9 studies, reported that the adjunctive application of HA yielded moderate but favorable clinical and radiographic outcomes in the surgical treatment of periodontal defects.[14] Suchetha et al. reported that the use of Gengigel significantly reduced the PI scores ($p < 0.001$), from baseline (2.19 ±0.41) to 1 month (1.33 ±0.17) and 3 months (1.10 ±0.07),[16] while in the present study, Gengigel showed a reduction of −0.51 ±0.33. The PPD (7.70 ±1.07 mm vs. 5.35 ±0.75 mm) and CAL (8.00 ±1.01 mm vs. 5.85 ±0.81 mm) scores observed by Suchetha et al. at baseline and a 90-day follow-up, respectively,[16] were higher than in the present trial. A significant reduction in the mean PPD between baseline (4.38 ±0.62 mm) and 6 weeks (3.19 ±0.75 mm), and then 3 months (2.81 ±0.66 mm) was noted in a study by Shergill et al.[26] In the current study, the mean difference in PPD from baseline to follow-up after the application of Gengigel was −0.16 ±0.04 mm. In our study, Gengigel also showed significant improvement in PI (−0.51 ±0.33), while a study conducted by Sahayata et al. reported non-significant improvement (3.16 ±0.42 vs. 2.30 ±0.36) when compared to placebo and scaling.[17] Kalra et al.[18] and Sandhu et al.[19] used Gengigel along with the amniotic membrane and platelet-rich fibrin, respectively, showing promising results. A study by Gupta et al. reported a mean reduction in PPD of −1.33 ±0.50 mm from baseline to 6 months, along with a reduction in PI and a mean gain in relative attachment level (RAL) of 1.78 ±0.83.[27] An RCT conducted on 14 patients by Fawzy El-Sayed et al. in 2012 showed that the application of hyaluronan gel in conjunction with periodontal surgery brought improvement in the PI, CAL (5.50 mm vs. 2 mm) and PPD (5 mm vs. 2 mm) values over time.[28] The outcomes observed in the present study might stem from the reduction of gingival inflammation, which led to the contraction and reconnection of connective tissue fibers, as well as a decrease in inflammatory mediators.

Addressing the global crisis of antibiotic resistance primarily involves minimizing antibiotic usage. Addressing the global crisis of antibiotic resistance primarily involves minimizing antibiotic use. Reactive oxygen species represent a promising alternative, as they can be administered directly to the affected area through topical application, thereby reducing the reliance on systemic antibiotics.[11] The notable reduction in PPD observed at the sites treated with BlueM gel is attributed to the increased release of active oxygen, which promotes rapid and progressive healing. Juliana and Tarek's research found that BlueM oxygen gel accelerated re-epithelialization, likely due to its role in promoting neovascularization, enhancing new blood vessel formation, and stimulating stem cell activity that supports fibroblast proliferation.[29] In a study by Koul et al., the reported reduction in PPD after using BlueM gel was 2–3 mm at 30 days,[9] which is less than in our study (−0.11 ±0.19). In the current investigation, BlueM gel demonstrated more significant efficacy in reducing CAL than the other 2 interventions. In a study conducted by Niveda and Kaarthikeyan,[30] the decrease in PPD observed from baseline (7.2 ±0.42 mm) to follow-up (4.7 ±0.57 mm) was greater than in the present investigation, where the mean reduction was −0.11 ±0.19. Research by Rajkhowa et al. showed that BlueM gel helped reduce both PI and OHI, and thus could be safely used to reduce the microbial load.[31] In a case report by Asha et al., BlueM gel was locally delivered for 7 days to treat periodontal pockets.[12] The patients were evaluated after 30 days, revealing a moderate reduction in PPD.[12]

Limitations

The study was conducted over a 6-month period with a relatively small sample size. This duration may be insufficient to comprehensively evaluate the effects of periodontal therapy, especially those involving regenerative techniques. Therefore, further research with a longer follow-up period and a larger sample size is warranted to assess the long-term stability of the outcomes.

Conclusions

The present study demonstrated that both BlueM gel and Gengigel are reliable adjuncts to SRP. Both agents effectively enhance periodontal health when used in combination with SRP, with Gengigel showing greater reductions in PPD, OHI and PI, while BlueM gel achieved superior improvement in CAL. Notably, this study is the first to assess the efficacy of both gels in this context. Future multicenter studies are needed to confirm and extend these findings across diverse populations and clinical settings. Additionally, comparative evaluations of this protocol against other established and clinically proven agents are warranted for a comprehensive assessment.

Ethics approval and consent to participate

The study was approved by the institutional review board (IRB) at MNR Dental College and Hospital, Sangareddy, India (MNR EC-BHR-07/23). The participants were fully briefed about the study and consented in writing.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

The authors of this manuscript declare that in the writing process of this work, no generative artificial intelligence (AI) or AI-assisted technologies were used to generate content, ideas or theories. We utilized Grammarly for the purpose of enhancing readability and refining language. This use was under strict human oversight and control. After the application of AI technologies, the authors carefully reviewed and edited the manuscript to ensure its accuracy and coherence.

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References

1. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet*. 2005;366(9499):1809–1820. doi:10.1016/S0140-6736(05)67728-8
2. Gontiya G, Galgali SR. Effect of hyaluronan on periodontitis: A clinical and histological study. *J Indian Soc Periodontol*. 2012;16(2):184–192. doi:10.4103/0972-124X.99260
3. Shaddox LM, Walker CB. Treating chronic periodontitis: Current status, challenges, and future directions. *Clin Cosmet Investig Dent*. 2010;2:79–91. PMID:23662085. PMCID:PMC3645457.
4. Gasner NS, Schure RS. Periodontal disease. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2023. Updated May 12, 2025. http://www.ncbi.nlm.nih.gov/books/NBK554590. Accessed January 3, 2024.
5. Mohammad CA, Mirza BA, Mahmood ZS, Zardawi FM. The effect of hyaluronic acid gel on periodontal parameters, pro-inflammatory cytokines and biochemical markers in periodontitis patients. *Gels*. 2023;9(4):325. doi:10.3390/gels9040325
6. Rajan P, Baramappa R, Rao NM, Pavaluri AK, Indeevar P, Ur Rahaman SM. Hyaluronic acid as an adjunct to scaling and root planing in chronic periodontitis. A randomized clinical trial. *J Clin Diagn Res*. 2014;8(12):ZC11–ZC14. doi:10.7860/JCDR/2014/8848.5237
7. Amato M, Santonocito S, Polizzi A, et al. Local delivery and controlled release drugs systems: A new approach for the clinical treatment of periodontitis therapy. *Pharmaceutics*. 2023;15(4):1312. doi:10.3390/pharmaceutics15041312
8. Knighton DR, Hunt TK, Scheuenstuhl H, Halliday BJ, Werb Z, Banda MJ. Oxygen tension regulates the expression of angiogenesis factor by macrophages. *Science*. 1983;221(4617):1283–1285. doi:10.1126/science.6612342
9. Koul A, Kabra R, Chopra R, Sharma N, Sekhar V. Comparative evaluation of oxygen releasing formula (Blue-M Gel®) and chlorhexidine gel as an adjunct with scaling and root planing in the management of patients with chronic periodontitis – a clinico-microbiological study. *J Dent Spec*. 2019;7(2):111–117. doi:10.18231/j.jds.2019.026
10. Blue®M. Our founding father: Dr. Peter Blijdorp. https://bluemcare.com/founding-father. Accessed January 4, 2024.
11. Deliberador TM, Weiss SG, Rychuv F, et al. Comparative analysis in vitro of the application of Blue®M oral gel versus chlorhexidine on *Porphyromonas gingivalis*: A pilot study. *Adv Microbiol*. 2020;10(4):194–201. doi:10.4236/aim.2020.104015
12. Asha A, Prabhuji ML, Rashmi P, Roja Y, Bhavikatti SK. Pocket reduction with high concentrated oxygen oral gel: A preliminary case report. *Res J Pharm Technol*. 2022;15(10):4367–4371. doi:10.52711/0974-360X.2022.00732
13. Bhati A, Fageeh H, Ibraheem W, Fageeh H, Chopra H, Panda S. Role of hyaluronic acid in periodontal therapy (Review). *Biomed Rep*. 2022;17(5):91. doi:10.3892/br.2022.1574
14. Rodríguez-Aranda M, Iborra-Badia I, Alpiste-Illueca F, López-Roldán A. Hyaluronic acid for periodontal tissue regeneration in intrabony defects. A systematic review. *Dent Rev*. 2022;2(3):100057. doi:10.1016/j.dentre.2022.100057
15. Gengigel®. About Gengigel. https://www.gengigel.co.uk/about-gengigel. Accessed January 4, 2024.
16. Suchetha A, Tanwar E, Sapna R, Darshan BM, Apoorva SM, Bhat D. Comparative evaluation of 0.2% hyaluronic acid gel (Gengigel®) and photodynamic therapy in the treatment of chronic periodontitis: A clinical study. *IJDSIR*. 2019;2(2):382–392. https://www.ijdsir.com/asset/images/uploads/1556383634766.pdf. Accessed April 6, 2024.
17. Sahayata VN, Bhavsar NV, Brahmbhatt NA. An evaluation of 0.2% hyaluronic acid gel (Gengigel®) in the treatment of gingivitis: A clinical an microbiological study. *Oral Health Dent Manag*. 2014;13(3):779–785. PMID:25284557.
18. Kalra SH, Monga C, Kalra KH, Kalra SH. A roentgenographic assessment of regenerative efficacy of bioactive Gengigel(®) in conjunction with amnion membrane in grade II furcation defect. *Contemp Clin Dent*. 2015;6(2):277–280. doi:10.4103/0976-237X.156068
19. Sandhu GK, Khinda PK, Gill AS, Kalra HS. Surgical re-entry evaluation of regenerative efficacy of bioactive Gengigel(®) and platelet-rich fibrin in the treatment of grade II furcation: A novel approach. *Contemp Clin Dent*. 2015;6(4):570–573. doi:10.4103/0976-237X.169855
20. Jain T, Kapoor A, Saini N, Chatterjee D, Jain S, Doriya JK. The new periodontal disease classification: Analysis and review. *University J Dent Scie*. 2021;7(3):137–141. doi:10.21276/ujds.2021.7.3.23
21. Manjunatha VA, Vemanaradhya GG, Gowda TM. Clinical and antioxidant efficacy of 4% mangosteen gel as a local drug delivery in the treatment of chronic periodontitis: A placebo-controlled, split-mouth trial. *Dent Med Probl*. 2022;59(1):111–119. doi:10.17219/dmp/139198

22. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1964;22:121–135. doi:10.3109/00016356408993968
23. Greene JC, Vermillion JR. The simplified oral hygiene index. *J Am Dent Assoc*. 1964;68:7–13. doi:10.14219/jada.archive.1964.0034
24. Wu CD, Savitt ED. Evaluation of the safety and efficacy of over-the-counter oral hygiene products for the reduction and control of plaque and gingivitis. *Periodontol 2000*. 2002;28:91–105. doi:10.1034/j.1600-0757.2002.280105.x
25. Ballini A, Cantore S, Capodiferro S, Grassi FR. Esterified hyaluronic acid and autologous bone in the surgical correction of the infra-bone defects. *Int J Med Sci*. 2009;6(2):65–71. doi:10.7150/ijms.6.65
26. Shergill SK, Sood R, Aggarwal R, Singh J, Sharma E, Jain S. Comparative evaluation of three commercially available local drug delivery medicaments in the treatment of chronic periodontitis. *Int J Oral Health Sci*. 2022;12(2):65–72. doi:10.4103/ijohs.ijohs_9_22
27. Gupta S, Kediege SD, Gupta A, Jain K. Evaluation of Gengigel® application in the management of furcation with coronally advanced flap through surgical re-entry – a split mouth clinical study. *J Clin Diagn Res*. 2017;11(1):ZC27–ZC32. doi:10.7860/JCDR/2017/21938.9169
28. Fawzy El-Sayed KM, Dahaba MA, Aboul-Ela S, Darhous MS. Local application of hyaluronan gel in conjunction with periodontal surgery: A randomized controlled trial. *Clin Oral Investig*. 2012;16(4):1229–1236. doi:10.1007/s00784-011-0630-z
29. Juliana H, Tarek S. Comparative study of the effect of BlueM active oxygen gel and coe-Pack dressing on postoperative surgical depigmentation healing. *Saudi Dent J*. 2022;34(4):328–334. doi:10.1016/j.sdentj.2022.04.005
30. Niveda R, Kaarthikeyan G. Effect of oxygen releasing oral gel compared to chlorhexidine gel in the treatment of periodontitis. *J Pharm Res Int*. 2020;32(19):75–82. doi:10.9734/jpri/2020/v32i1930711
31. Rajkhowa C, Supriya SL, Bhavadharani J, Jannath AA, Nivedha K, Padmanaban A. Comparison of the efficacy of active oxygen-containing mouthwash (BlueM) with chlorhexidine mouthwash (Hexidine) and its effect on plaque, calculus, and gingival inflammation. *Int J Oral Health Dent*. 2023;9(1):44–47. doi:10.18231/j.ijohd.2023.008

Original paper

Occurrence of dental caries, the prevalence of hygiene and dietary habits, and the influence of the workout session on the selected parameters of saliva in youth swimmers

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Abstract

Background. Poor oral health can affect the overall health of the body, which may result in suboptimal performance in sports.

Objectives. The aim of the present study was to evaluate the occurrence of tooth decay, the prevalence of hygiene and dietary habits, and the influence of the workout session on the selected salivary parameters in youth swimmers.

Material and methods. The study involved 40 adolescents, aged 12–15 years (mean age: 13 ±0.5 years), including 30 competitive swimmers and 10 controls. The decayed, missing and filled teeth index (DMFT) was assessed. Data on oral hygiene and dietary habits was collected through a questionnaire created by the authors. Salivary samples were collected from all subjects in the morning and in the afternoon, and from swimmers before and after training. The salivary flow rate (V), pH, buffering capacity, and calcium (Ca) level were measured.

Results. The DMFT in the group of swimmers was 4.5 ±3.54, which was significantly higher than in the control group (1.6 ±2.59) ($p = 0.0007$). There were no statistically significant differences in oral hygiene habits between the 2 groups. However, swimmers significantly more often consumed chocolate bars ($p = 0.004$), energy drinks ($p = 0.017$) and water ($p = 0.002$) as compared to the control group. Among the consumed beverages, significantly more sweet carbonated drinks were consumed by swimmers than controls ($p = 0.026$). After the workout session, both in the morning and in the afternoon, there was a significant decrease in V ($p = 0.006$ and $p = 0.009$, respectively) and a significant increase in the Ca level ($p = 0.001$ and $p = 0.001$, respectively) in swimmers. While comparing swimmers before training with the control group in the morning, significantly higher V ($p = 0.012$) and lower pH ($p = 0.003$) were observed in swimmers. In the afternoon, significantly lower pH was found in swimmers ($p = 0.006$).

Conclusions. Oral health prevention and promotion should be an important aspect of sports participation, through greater awareness of the impact of oral hygiene and diet on overall health, which can influence sports performance.

Keywords: dietary habits, dental caries, saliva, oral hygiene, physical exertion

Highlights

- Athletes' oral health should be prioritized during sports training and competition.
- Swimmers tend to consume carbohydrate-rich foods and drinks more frequently than the general population, which correlates with a higher incidence of dental caries.
- After a swimming training session, swimmers experience a significant decrease in the salivary flow rate and a significant increase in the calcium level.
- Raising awareness about the importance of oral health monitoring is essential to understand its impact on overall health and sports performance.

Introduction

In accordance with the guidelines developed by the World Dental Federation (FDI), a healthy oral cavity is an important factor in helping athletes achieve their best performance, and conversely, poor oral health affects overall well-being and can have a negative impact on sports performance.[1] Achieving good results in sports serves as motivation for further training to improve performance. Sudden, unexpected problems in the oral cavity can result in the interruption of systematically conducted training for sports competitions, and even withdrawal from them. This is confirmed in a study by Needleman et al., in which athletes emphasized long periods of inability to train and the lack of exercise at the desired level due to oral health problems.[2] Therefore, the oral health of athletes should be considered a priority during sports activities.[3] Athletes themselves often have a dismissive attitude toward oral health.[4] From the few published works, it is evident that poor oral health can have a negative impact on sports performance and training.[2,5–7] Among individuals engaged in regular sports training, the most common oral health issues are tooth decay and gum inflammation, affecting over 50% of athletes.[2,5–7] Other common conditions among athletes include tooth injuries and erosion.

The decayed, missing and filled teeth index (DMFT) is a valuable indicator used for determining and monitoring the oral health status in a community. Moreover, it is the most important index used in epidemiological studies.[8] It determines the number of decayed teeth (DT), the number of teeth missing due to decay (MT) and the number of treated teeth (FT). The DMFT is used to evaluate and monitor oral health interventions in a community by developing policies and programs related to this area.[9–12]

Caries is a multifactorial dental disease and is one of the most common global health problems.[13] Bacteria in the plaque metabolize carbohydrates from the diet into acids, leading to a decrease in pH in the oral cavity, and thus causing the demineralization of the enamel and root cementum.[14]

The main causes of poor oral health among athletes include negligence related to oral hygiene, a poor diet, an unhealthy lifestyle, and lowered immunity.

Poor oral hygiene is the primary factor in the development of dental caries.

It is well known that frequent consumption of carbohydrates leads to a decrease in pH in the oral environment, causing the demineralization of the teeth and the subsequent development of carious lesions.[6] Many athletes choose carbohydrate-rich foods because of their ability to increase energy levels during training. Foods that adhere to the tooth surface further increase the chance of lowering oral pH, causing a shift in the balance toward the demineralization process. Examples of such often chosen foods are candy bars and muesli. Athletes also tend to choose acidic fruits as snacks during exercise, like oranges, which contributes to the loss of minerals from the tooth tissue.[15]

Saliva is responsible for maintaining proper homeostasis in the oral cavity.

The average daily volume of saliva secretion is 0.5–2 L, and depends on the amount of sleep, the frequency and type of meals, and emotional stimuli.[16] The rate of saliva secretion under normal conditions, in the absence of external stimulation, varies from 0.25 mL/min to 0.35 mL/min, which can increase to 1.50–2.30 mL/min under the influence of a food stimulus.[17] In contrast, during sleep, when the process of saliva secretion is slower, it decreases to 0.05 mL/min.[18]

Saliva has the buffering (acid neutralization) capacity and its diluting action regulates pH in the oral environment. The constant flow of saliva removes food debris and microorganisms from the oral cavity. The ability to remineralize hard dental tissues is due in part to the presence of the calcium (Ca), phosphate and fluoride ions in saliva.[19,20]

Along with blood, saliva can be an alternative diagnostic material for monitoring the response of the body to exercise.[21] The advantage of saliva as a diagnostic material over blood is the non-invasive way it is collected. Stress that often occurs during blood collection due to the sight of a needle or pain during insertion can alter the results of the parameters tested. Saliva secretion is regulated by the autonomic nervous system.[22] Physical exercise induces changes in the activity of the autonomic nervous system, which leads to changes in saliva secretion as well as its composition.

Therefore, the intensity, type, course, duration, and time of day of exercise are crucial in altering salivary parameters.[23] Decreased salivary secretion during intense exercise is explained by an increase in sympathetic innervation activity, causing the constriction of the vessels supplying blood to the salivary glands, and consequently lowering the saliva volume, while the activity of the parasympathetic system increases it.[24] Also, physical exercise causes an increase in vasopressin (an antidiuretic hormone), which can decrease the production of saliva. The effect of the dehydration of the body during prolonged exercise and the evaporation of saliva following mouth breathing, as well as hyperventilation, cannot be overlooked either.[25] Saliva as a diagnostic tool, in particular, determining its rate of secretion, buffering capacity, pH value, and the growth of caries-forming bacteria, is applied for assessing the caries risk.[13,16,26]

Although more and more research on the monitoring of effort through the analysis of biochemical changes in saliva is being conducted, unfortunately, little information is available on the relationship between salivary parameters and oral health in adolescents who train in sports.

The objective of the present study was to determine the occurrence of dental caries, the prevalence of hygiene and dietary habits, and the influence of the training session on the selected salivary parameters in youth swimmers.

Material and methods

The study included 40 adolescents, aged 12–15 years (mean age: 13 ±0.5 years), of which 30 were competitive swimmers (the study group) and 10 were sedentary individuals (the control group). Swimmers were recruited from 2 swimming championship clubs operating in the city, and all of them (n = 30) participated in the study. The control group (n = 10) consisted of clinically healthy participants who were not taking any medications based on their medical history, did not participate in sports competitions, played sports only recreationally, and were age- and gender-matched with swimmers.

The clinical examination was carried out using a standard mirror and a dental probe under artificial lighting. The examination involved the assessment of the condition of the tooth tissue in terms of carious disease.

The condition of the teeth was assessed according to the World Health Organization (WHO) criteria based on the DMFT index.[27] The DMFT was calculated for each subject, as well as the prevalence of caries in both groups.

All subjects completed a questionnaire created by the authors for the purpose of the study. The questions regarded the sports the participants played, the frequency and time of day of tooth brushing, and the type of toothbrush they used, as well as the date of their last dental visit and the reason for it. In addition, the respondents provided information regarding the consumption of sugary snacks, the type of snacks and the amount of liquids consumed during the day. Also, swimmers provided information on the type and amount of drinks consumed after training. The questionnaire did not exclude the normal consumption of high-carbohydrate snacks beyond training in either group, but differentiated the athletes group from the control group during peri-workout time.

Swimmers trained 21 h a week in the morning (6:00–7:45 a.m.) and in the afternoon (4:00–5:45 p.m.). The swimming workout session consisted of swimming a distance of 5,000 meters. All the recruited participants had to provide written informed parental consent, be willing to undergo saliva sampling and answer the questionnaire. Participants who did not meet the inclusion criteria were excluded from the study.

The study protocol was approved by the Bioethics Committee at Wroclaw Medical University, Poland (approval No. KB-327/2009). All examinations were conducted in accordance with the relevant guidelines and regulations.

Saliva sampling and the studied parameters

Unstimulated mixed saliva samples were collected from swimmers 4 times – before and after workout, in the morning and in the afternoon. In the control group, the samples were collected twice, at the time of the pre-workout sampling in swimmers. All test subjects were asked to rinse their mouths with water before saliva collection. In total, 140 samples of saliva were collected daily. During sampling, the subjects sat with their heads bowed and their mouths open. Saliva samples were collected from the floor of the mouth, with the use of a plastic pipette, and put into a graded test tube stored on crushed ice. Based on the measurement of the volume of the collected saliva sample and the time needed for its collection, the salivary flow rate (V) was calculated and expressed in mL/min.

The samples were centrifuged for 10 min at a speed of 3,500 rpm before chemical assays. The pH and the buffering capacity in mmol/L (the potentiometric method), and the Ca level (the method based on the formation of a chromogenic complex between the Ca ions and o-cresolphthaelin, using a commercial kit (Alpha Diagnostics, Warsaw, Poland)) were assessed.

Statistical analysis

Depending on variable distribution, Student's t test or the Mann–Whitney U test were used for the analysis of independent variables. For dependent variables, repeated measures Student's t test or non-parametric repeated measures Wilcoxon's test were employed. Moreover, Fisher's test was used if the data was categorical (answers to questions). To study the monotonic component of the relationship between the variables, Spearman's coefficient

of correlation (ρ) between the DMFT scores and the difference in salivary parameters before and after training, as well as with hygiene and dietary habits, was calculated. In addition, the correlation between dietary habits and salivary parameters was checked.

The level of significance was set at $p < 0.05$. All analyses were performed with the use of the Statistica 13.3 software package (StatSoft, Inc., Tulsa, USA) and the R package (https://www.r-project.org).

In the statistical analysis, we employed an exploratory approach, without including a correction for the families of hypotheses, which leads to an increased risk of type I error. Thus, the results must be interpreted with caution. The study might serve as a starting point for further research in the field.

Results

DMFT ratio and caries prevalence

Caries prevalence in swimmers was 56.7%, and it was 40.0% in the control group (Fig. 1).

In the studied athletes, the DMFT values were as follows: DT = 1.2; MT = 0.0; and FT = 3.3. In contrast, in the control group, the mean values of the components of DMFT were DT = 0.8, MT = 0.0 and FT = 0.8. Overall, DMFT in the studied athletes was 4.5, while in the control group it was 1.6. In the group of swimmers, the FT value, as well as the total DMFT value, were significantly higher as compared to the control group ($p = 0.0005$ and $p = 0.0007$, respectively). The mean and median values for the DT, MT, FT, and DMFT variables are shown in Table 1.

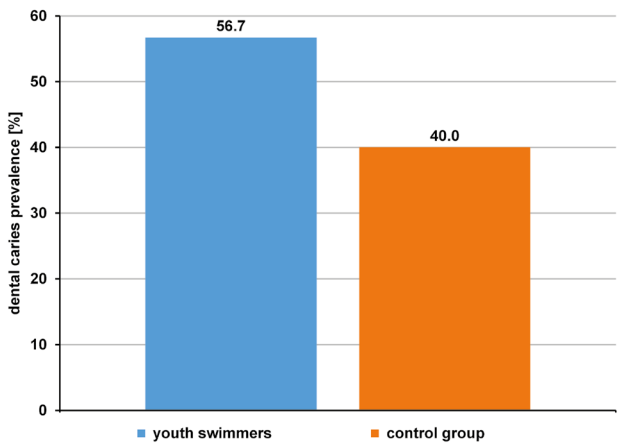

Fig. 1. Dental caries prevalence in youth swimmers and the control group

Frequency and time of tooth cleaning, and the type of toothbrush used

No statistically significant differences in oral hygiene habits were observed between the 2 groups. Most of the subjects, both swimmers and controls, brushed their teeth twice a day (66.7% vs. 80.0%); 16.7% of swimmers reported brushing their teeth more often than twice a day, while 13.3% brushed only once a day. In the morning, 60.0% of the surveyed swimmers cleaned their teeth before breakfast, while 70% of controls cleaned their teeth after breakfast. Both swimmers (93.3%) and control group subjects (100.0%) cleaned their teeth in the evening. Both groups (86.7% vs. 100.0%, respectively) used a manual toothbrush (Table 2).

Eating habits

Almost all swimmers declared that they did not follow a special diet for athletes (96.6%), and only one person (3.4%) followed such a diet. In the control group, 100.0% of the respondents followed a regular diet.

Swimmers consumed carbohydrate-containing products more often as compared to the control group. Most of them (63.3%) consumed sweet rolls, doughnuts or cookies every day or almost every day. In contrast, chips were not very popular in either group and were consumed occasionally (43.4% in the swimmer group vs. 30.0% in the control group). Most swimmers consumed chocolate bars daily or nearly daily (80.0%). In the control group, only 30.0% consumed this snack on a daily basis. The difference

Table 1. The DMFT (decayed (DT), missing (MT) and filled (FT) teeth) values for swimmers and the control group

Dental status	Swimmers (n = 30)						Control group (n = 10)						p-value
	M ±*SD*	*Me*	min	max	lower quartile	upper quartile	*M* ±*SD*	*Me*	min	max	lower quartile	upper quartile	
DT	1.2 ±1.27	1.00	0.00	4.00	0.00	2.00	0.8 ±1.55	0.00	0.00	5.00	0.00	1.00	0.935
MT	0.0 ±0.00	0.00	0.00	0.00	0.00	0.00	0.0 ±0.00	0.00	0.00	0.00	0.00	0.00	–
FT	3.3 ±3.19	3.00	0.00	12.00	1.00	4.00	0.8 ±1.13	0.00	0.00	3.00	0.00	2.00	0.0005*
DMFT	4.5 ±3.54	4.00	0.00	15.00	2.00	6.00	1.6 ±2.59	0.00	0.00	8.00	0.00	3.00	0.0007*

M – mean; *SD* – standard deviation; *Me* – median; min – minimum; max –maximum; * statistically significant.
DMFT = (DT + MT + FT) / number of examined people

Table 2. Oral hygiene habits of swimmers and the control group

Oral habit		Swimmers (*n* = 30)	Control group (*n* = 10)
When do you clean your mouth?	before breakfast	18 (60.0)	4 (40.0)
	after breakfast	16 (53.3)	7 (70.0)
	before supper	2 (6.7)	0 (0.0)
	before sleep	28 (93.3)	10 (100.0)
How often do you clean your mouth?	once a day	4 (13.3)	1 (10.0)
	twice a day	20 (66.7)	8 (80.0)
	more than twice a day	5 (16.7)	0 (0.0)
	after eating	0 (0.0)	1 (10.0)
	every second day	0 (0.0)	0 (0.0)
	once for a few days	0 (0.0)	0 (0.0)
	as I recall	1 (3.3)	0 (0.0)
What kind of toothbrush do you use?	always manual	26 (86.7)	10 (100.0)
	always electric	1 (3.3)	0 (0.0)
	manual and electric toothbrush	3 (10.0)	0 (0.0)
When was the last time you went to the dentist?	a month ago	14 (46.7)	2 (20.0)
	3 months ago	2 (6.7)	2 (20.0)
	half a year ago	7 (23.3)	5 (50.0)
	a year ago	4 (13.3)	1 (10.0)
	I don't remember	3 (10.0)	0 (0.0)
What was the reason for the visit?	noticed carious lesion	6 (20.0)	2 (20.0)
	dental pain	1 (3.3)	0 (0.0)
	prevention	0 (0.0)	2 (20.0)
	check-up	23 (76.7)	6 (60.0)

Data presented as number (percentage) (*n* (%)).

between the groups proved to be statistically significant in this regard (p = 0.004). Also, candies were consumed very often by swimmers, while in the control group, daily consumption was recorded in only one person (26.7% vs. 10.0%, respectively). Among control group subjects, 70.0% consumed this snack occasionally. The situation was similar with the consumption of chocolate, which was eaten daily or nearly daily by most swimmers as compared to the control group (93.3% vs. 40.0%) (Fig. 2).

In addition to sweet snacks, the diet of both groups contained sweet drinks. Swimmers consumed energy drinks significantly more often (p = 0.017) (Fig. 3) and drank more sweet carbonated drinks per day (p = 0.026) (Fig. 4) than the control group. Both groups reported daily water consumption (100.0% of swimmers vs. 60.0% of control subjects), with swimmers consuming this beverage significantly more often (p = 0.002) (Fig. 3).

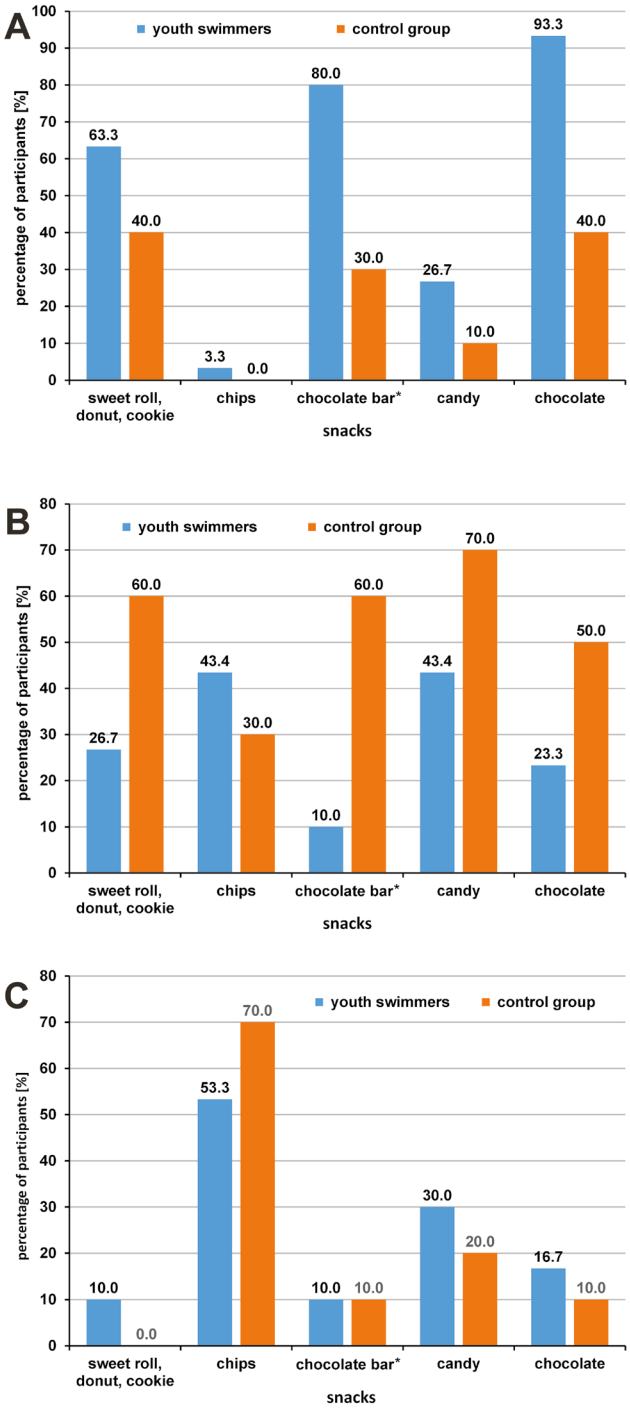

Fig. 2. Types of carbohydrate-containing products (snacks) consumed by youth swimmers and the control group with regard to the frequency of consumption

A – every day or almost every day; B – occasionally; C – never or almost never.
* p = 0.004.

Drinks consumed after exercise

Every day, after each swim workout, most swimmers (80.0%) consumed water, followed by fruit drinks (63.3%), vitaminized drinks (43.4%) and energy drinks (20.0%). Swimmers consumed fruit drinks (p = 0.032) and vitaminized drinks (p = 0.023) after exercise significantly more often as compared to other beverages (Fig. 5).

Also, swimmers drank significantly more vitaminized drinks after the workout session (p = 0.016) (Fig. 6).

Changes in the studied saliva parameters according to the time of day

In swimmers, before and after training in the morning, we found a lower V value and higher Ca content than in the samples taken in the afternoon. After training, we observed lower pH levels in the morning than in the afternoon. The buffering capacity before the morning workout was lower as compared to afternoon training. However, in the afternoon, the results were the opposite after swimming training. In the control group, we observed lower V and buffering capacity, as well as a higher Ca level, in the morning as compared to the afternoon, and the pH value was the same in the morning and in the afternoon (Table 3).

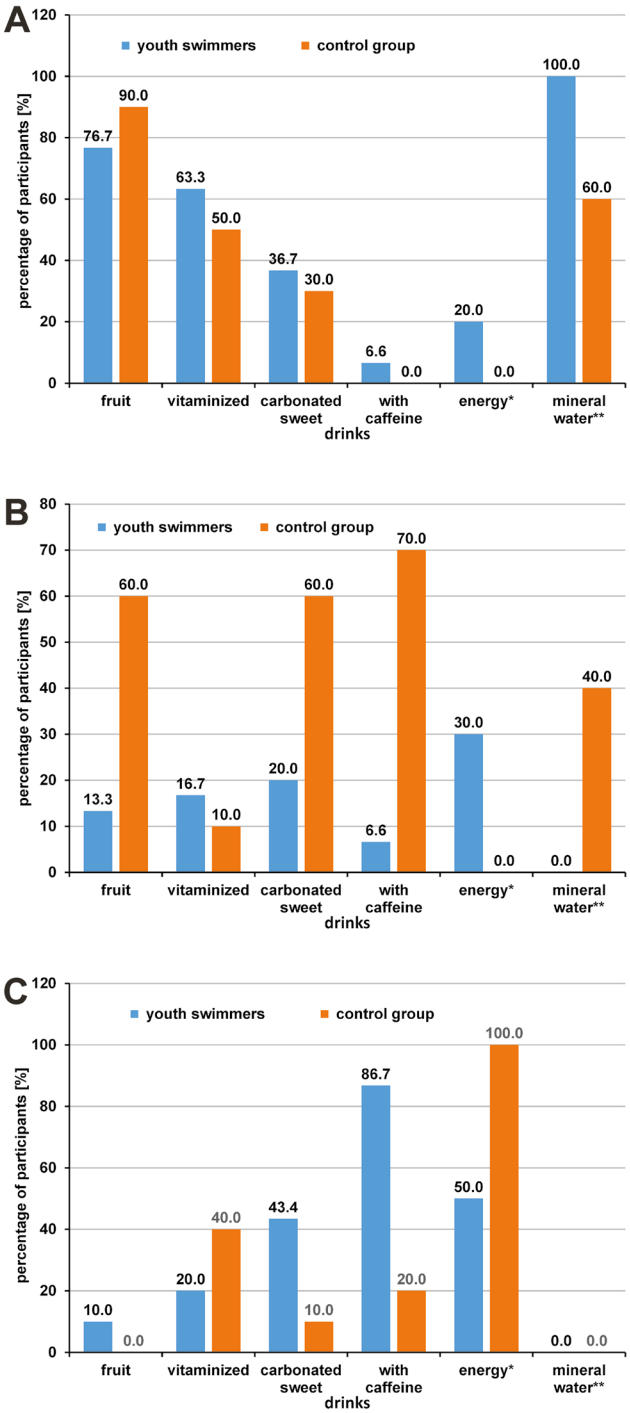

Fig. 3. Types of drinks consumed by youth swimmers and the control group with regard to the frequency of consumption

A – every day or almost every day; B – occasionally; C – never or almost never. * p = 0.017; ** p = 0.002.

Table 3. Salivary parameters of the samples collected from swimmers and non-swimmers

Salivary parameter	Time of day	Non-swimmers (control group)	Swimmer study group		Swimmers before training vs. non-swimmers	Swimmers before vs. after training
			before training	after training		
V [mL/min]	a.m.	0.26 ±0.15	0.47 ±0.37	0.36 ±0.22	$u = 2.499$ $p = 0.012^{b*}$ (↑ 45%)	$z = 2.746$ $p = 0.006^{c*}$ (↓ 23%)
	p.m.	0.35 ±0.19	0.63 ±0.63	0.49 ±0.39	$u = 1.812$ $p = 0.070^{b}$ (↑ 44%)	$z = 2.606$ $p = 0.009^{c*}$ (↓ 22%)
	a.m. vs. p.m.	$z = 1.376$ $p = 0.168^{c}$ (↑ 26%)	$z = 2.417$ $p = 0.016^{c*}$ (↑ 25%)	$z = 2.931$ $p = 0.003^{c*}$ (↑ 26%)	–	–
pH	a.m.	7.53 ±0.33	7.01 ±0.46	7.15 ±0.33	$t = -3.192$ $p = 0.003^{a*}$ (↓ 7%)	$t = -1.713$ $p = 0.097^{a}$ (↑ 2%)
	p.m.	7.53 ±0.49	7.13 ±0.33	7.27 ±0.24	$t = -2.932$ $p = 0.006^{a*}$ (↓ 5%)	$t = -2.647$ $p = 0.013^{a*}$ (↑ 2%)
	a.m. vs. p.m.	$t = 0.729$ $p = 0.486^{a}$ (const. 0%)	$t = -1.597$ $p = 0.121^{a}$ (↑ 1.6%)	$t = -2.060$ $p = 0.048^{a*}$ (↑ 1.6%)	–	–
Buffering capacity [mmol/L]	a.m.	4.29 ±1.94	4.23 ±2.04	4.53 ±1.90	$u = -0.250$ $p = 0.802^{b}$ (↓ 1%)	$z = 0.182$ $p = 0.855^{c}$ (↑ 7%)
	p.m.	4.50 ±2.25	4.98 ±2.63	4.48 ±1.90	$u = 0.765$ $p = 0.444^{b}$ (↑ 10%)	$z = 1.278$ $p = 0.201^{c}$ (↓ 10%)
	a.m. vs. p.m.	$z = 0.770$ $p = 0.441^{c}$ (↑ 5%)	$z = 1.326$ $p = 0.184^{c}$ (↑ 15%)	$z = 0.746$ $p = 0.455^{c}$ (↓ 1%)	–	–
Ca level [mg/L]	a.m.	3.25 ±0.79	3.83 ±1.33	4.99 ±2.24	$t = 1.307$ $p = 0.199^{a}$ (↑ 15%)	$t = -4.182$ $p = 0.001^{a*}$ (↑ 23%)
	p.m.	3.18 ±0.96	3.72 ±1.34	4.61 ±1.58	$t = 1.191$ $p = 0.241^{a}$ (↑ 15%)	$t = -4.439$ $p = 0.001^{a*}$ (↑ 19%)
	a.m. vs. p.m.	$t = 0.185$ $p = 0.856^{a}$ (↓ 2%)	$t = 0.440$ $p = 0.663^{a}$ (↓ 3%)	$t = 0.890$ $p = 0.376^{a}$ (↓ 8%)	–	–

V – salivary flow rate; Ca – calcium; * statistically significant ([a] Student's t test, [b] Mann–Whitney U test, [c] Wilcoxon's test).

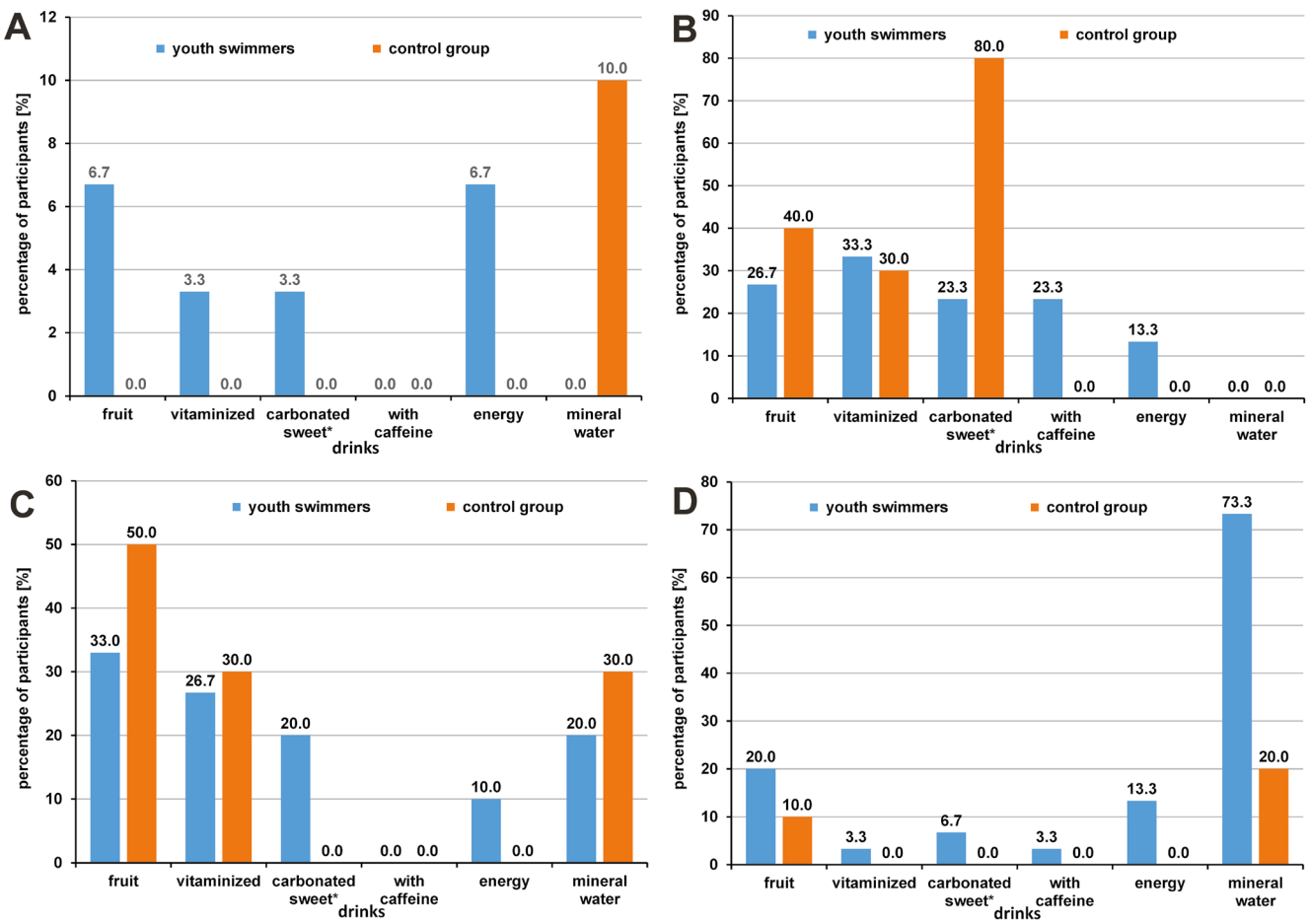

Fig. 4. Types and amount of drinks consumed per day by youth swimmers and the control group

A – can; B – 0.3-liter bottle; C – 0.5-liter bottle; D – 1-liter bottle.
* $p = 0.026$.

Saliva parameters in swimmers before training compared to the control group

In comparison with the control group, swimmers had higher V value and Ca level, and lower pH before training, which was performed both in the morning and in the afternoon. A lower buffering capacity was observed in the morning as compared to the control group, but a higher buffering capacity was observed in the afternoon as compared to the control group (Table 3).

Saliva parameters before and after the training session

After training, both in the morning and in the afternoon, V showed a significant decrease ($p = 0.006$ and $p = 0.009$, respectively), while there were increases in pH and the Ca level. The pH value was significantly higher after training in the afternoon ($p = 0.013$), and for the Ca content, the increase was significant both in the morning ($p = 0.001$) and in the afternoon ($p = 0.001$). Moreover, there was an increase in the buffering capacity after morning training, and a decrease after afternoon training (Table 3).

Correlations between changes in salivary parameters, hygiene and dietary habits and DMFT

With regard to the relationship between changes in salivary parameters and the DMFT scores, there was only a significant positive correlation with V in the morning before exertion in youth swimmers ($\rho = 0.448$; $p = 0.010$). As V increased, DMFT increased as well. However, in the control group there was no correlation between salivary parameters and DMFT in the morning. Such a result could be explained by the diet used. Therefore, the correlations between DMFT and hygiene and dietary habits in swimmers were checked. There was no correlation between DMFT and hygiene habits. However, the correlation came out positive for the consumption of sweet rolls, donuts and cookies, as well as chocolate bars ($\rho = 0.747$; $p = 0.000$ and $\rho = 0.408$; $p = 0.003$, respectively). As the frequency of consumption of these sweet snacks increased, DMFT increased as well. On the other hand, in the case of post-exercise beverage consumption, it was observed that DMFT increased as the frequency of energy drink consumption raised ($\rho = 0.375$; $p = 0.044$).

Correlation between changes in salivary parameters and dietary habits

Since a positive correlation was found between V and DMFT, the correlations between the studied salivary parameters and dietary habits in athletes were checked.

Taking into account differences in the pre- and post-training values of salivary parameters in the morning relative to dietary habits, there was a significant positive correlation between V and the frequency of consumption of sweet rolls, donuts and cookies (ρ = 0.551; p = 0.001 and ρ = 0.503; p = 0.004, respectively). The negative correlation between the consumption of carbohydrate-containing snacks and the salivary Ca level, before and after training in the morning is also noteworthy; as the frequency of consumption of sweet rolls, donuts and cookies increased, the Ca level decreased (ρ = −0.383; p = 0.036 and ρ = −0.369; p = 0.044, respectively).

Other significant Spearman's correlations, both positive and negative, before and after morning and afternoon training, are highlighted in Table 4.

Table 4. Significant Spearman's correlation between the salivary parameter values, before and after the workout session in the morning and in the afternoon, and dietary habits in swimmers

Carbohydrate-containing products	Saliva parameters	Time of day							
		morning				afternoon			
		before training		after training		before training		after training	
		ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value
Sweet roll, donut, cookie	V	0.551	0.001*	0.503	0.004*	0.340	0.065	0.212	0.260
	pH	0.212	0.259	0.098	0.605	0.306	0.099	0.038	0.839
	buffering capacity	−0.071	0.708	−0.134	0.479	−0.144	0.446	−0.077	0.685
	Ca	−0.383	0.036*	−0.369	0.044*	−0.159	0.339	−0.071	0.707
Chocolate bar	V	0.387	0.034*	0.183	0.332	0.023	0.901	0.045	0.812
	pH	0.118	0.533	0.360	0.050	0.207	0.270	0.053	0.779
	buffering capacity	−0.389	0.033*	0.102	0.588	−0.122	0.517	0.139	0.461
	Ca	−0.067	0.724	−0.095	0.614	0.060	0.751	−0.058	0.759
Fruit drink	V	−0.081	0.667	−0.055	0.769	−0.375	0.040*	−0.020	0.916
	pH	−0.113	0.552	−0.044	0.815	−0.374	0.041*	−0.348	0.059
	buffering capacity	0.101	0.593	−0.131	0.489	−0.129	0.494	−0.048	0.797
	Ca	−0.001	0.996	−0.152	0.421	−0.081	0.668	−0.084	0.656
Carbonated sweet drink	V	0.155	0.412	0.183	0.331	0.024	0.898	−0.081	0.667
	pH	0.049	0.793	−0.179	0.342	0.100	0.598	0.036	0.847
	buffering capacity	0.085	0.654	−0.113	0.550	−0.117	0.536	−0.493	0.005*
	Ca	−0.234	0.212	−0.265	0.156	−0.126	0.505	−0.333	0.071
Drink with caffeine	V	0.140	0.459	0.005	0.976	0.024	0.898	0.028	0.881
	pH	0.261	0.163	0.420	0.020*	0.095	0.616	0.285	0.126
	buffering capacity	−0.297	0.110	0.008	0.964	−0.155	0.410	−0.267	0.152
	Ca	−0.184	0.328	0.064	0.735	0.049	0.795	−0.099	0.600
Energy drink	V	0.283	0.128	0.435	0.016*	0.253	0.175	0.228	0.224
	pH	0.368	0.045*	0.196	0.296	0.134	0.477	0.223	0.234
	buffering capacity	0.035	0.853	−0.413	0.023*	0.339	0.066	−0.011	0.952
	Ca	−0.378	0.039*	−0.437	0.015*	−0.138	0.465	−0.127	0.501

ρ – Spearman's rank correlation coefficient; * statistically significant.

Correlation between changes in salivary parameters and the drinks consumed after swimmer training in the morning and in the afternoon

With regard to the relationship between changes in the post-training values of salivary parameters and the drinks drunk after swimmer training, both in the morning and in the afternoon, there was a significant positive correlation between V and the frequency of the consumption of sweet carbonated drinks (ρ = 0.398; p = 0.029 and ρ = 0.364; p = 0.047, respectively) and energy drinks (ρ = 0.666; p = 0.000 and ρ = 0.531; p = 0.002, respectively). However, there was a significant positive correlation between the frequency of consumption of caffeinated beverages and salivary pH after training in the afternoon (ρ = 0.425; p = 0.019), and a negative correlation for sweet carbonated beverages and the saliva buffering capacity after exercise (ρ = −0.411; p = 0.020) (Table 5).

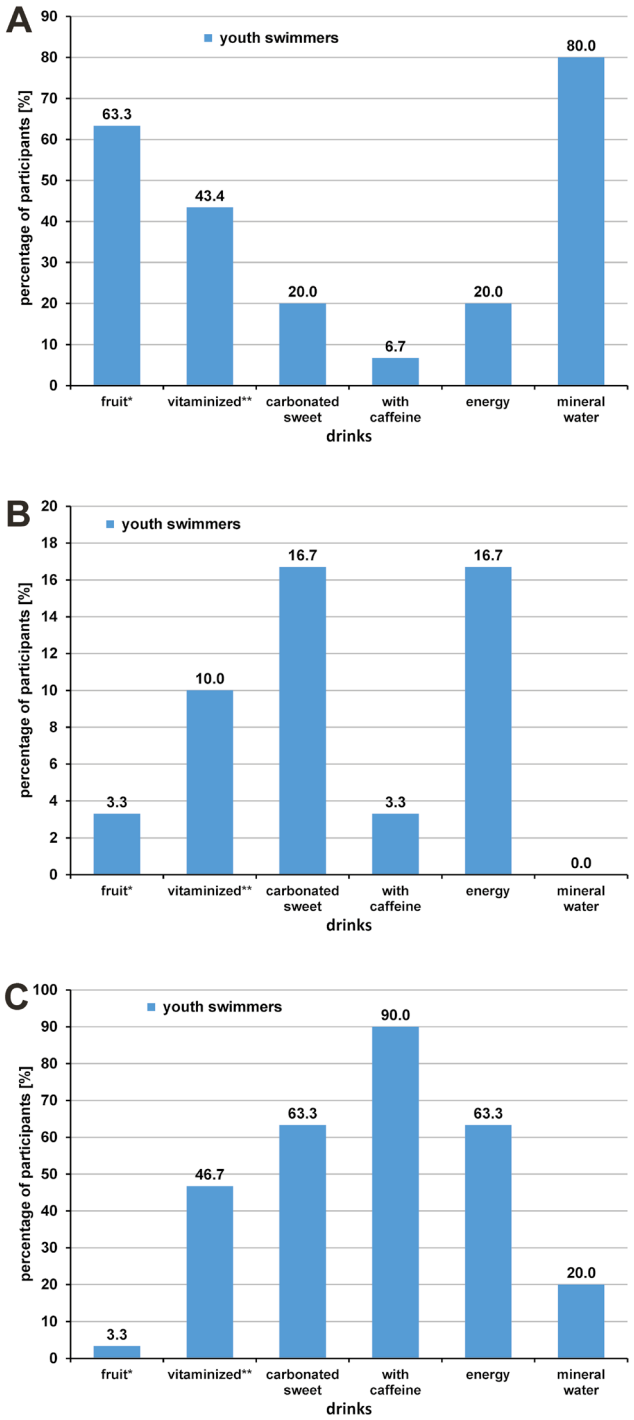

Fig. 5. Types of drinks consumed by youth swimmers after the workout session with regard to the frequency of consumption

A – every day or almost every day; B – occasionally; C – never or almost never. * p = 0.032; ** p = 0.023.

Table 5. Significant Spearman's correlation between the salivary parameter values and the drinks consumed by swimmers after the workout session in the morning and in the afternoon

Drinks consumed after training	Saliva parameters	Time of day			
		morning		afternoon	
		ρ	p-value	ρ	p-value
Fruit drink	V	−0.267	0.152	−0.010	0.954
	pH	0.012	0.949	−0.308	0.096
	buffering capacity	0.064	0.736	0.085	0.653
	Ca	−0.138	0.465	−0.042	0.825
Vitaminized drink	V	0.057	0.761	0.190	0.313
	pH	−0.011	0.952	−0.126	0.506
	buffering capacity	0.242	0.197	0.045	0.811
	Ca	0.177	0.349	0.233	0.214
Carbonated sweet drink	V	0.398	0.029*	0.364	0.047*
	pH	0.069	0.716	0.181	0.338
	buffering capacity	0.037	0.842	−0.411	0.023*
	Ca	−0.162	0.391	−0.185	0.325
Drink with caffeine	V	0.137	0.470	0.132	0.486
	pH	0.354	0.054	0.425	0.019*
	buffering capacity	0.191	0.310	−0.238	0.204
	Ca	−0.124	0.510	−0.076	0.686
Energy drink	V	0.666	0.000*	0.531	0.002*
	pH	0.313	0.091	0.338	0.067
	buffering capacity	−0.197	0.294	−0.054	0.776
	Ca	−0.244	0.192	−0.343	0.062
Mineral water	V	−0.085	0.654	0.069	0.717
	pH	−0.017	0.927	0.224	0.232
	buffering capacity	0.002	0.991	−0.067	0.723
	Ca	0.196	0.298	−0.011	0.950

ρ – Spearman's rank correlation coefficient; * statistically significant.

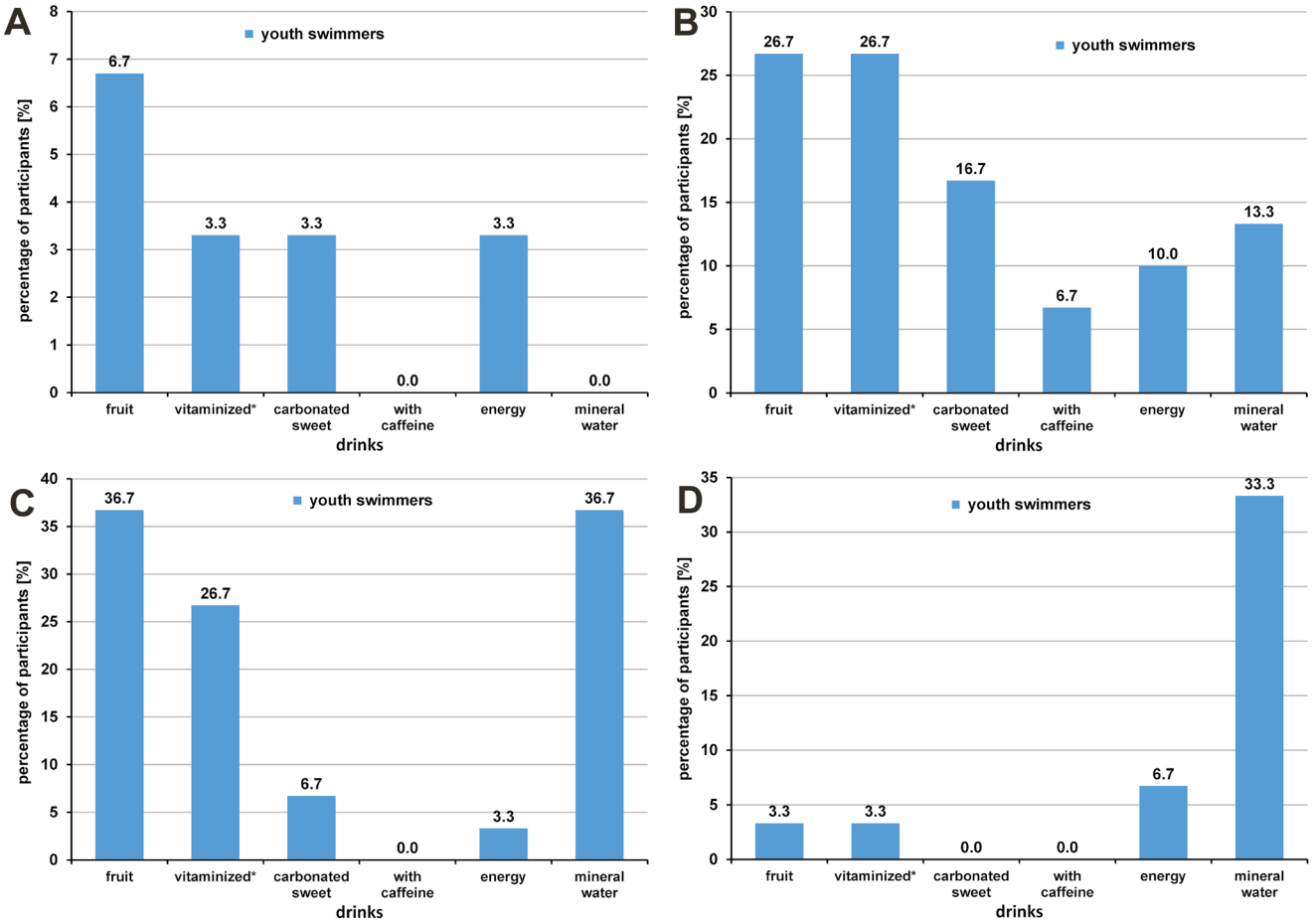

Fig. 6. Types and amount of drinks consumed by youth swimmers after the workout session

A – can; B – 0.3-liter bottle; C – 0.5-liter bottle; D – 1-liter bottle.
* $p = 0.016$.

Discussion

The results regarding carious disease prevalence, as defined by the DT number, showed that an average of 1.2 teeth in swimmers and 0.8 in the control group were affected by the carious process. The MT number was 0 in both groups. On the other hand, the average number of filled teeth (FT) was 3.3 and 0.8, respectively. The average DMFT score in the 2 groups differed, and was 4.5 for swimmers and 1.6 for the control group. The prevalence of caries in the study group of athletes was 56.7%, and 40.0% in the control group. The results of a study by Kaczmarek, conducted among swimmers aged 13–43 years, were as follows: the average DMFT score was 4.71, while in the control group it was 5.32.[6] The author also reported the values of the components of the index, with DT = 1.42, MT = 0.19 and FT = 3.10. In contrast, the prevalence of caries in both groups was similar, at 54.8% vs. 53.6%.[6] In the case of the elite athletes participating in the 2012 London Olympics, caries was found in 55% of them.[2] Gallagher et al., studying elite athletes aged 18 and above from various sports, along with swimming, found that 49.1% had teeth involved in caries.[28] A similar study assessing oral health was conducted by D'Ercole et al. in young swimmers (mean age: 12.5 years) compared to those not regularly involved in swimming (mean age: 9.85 years).[29] The researchers found tooth decay in 38.24% of competitive swimmers, and 42.86% in the non-competitive group.[29] Another study found that in non-sports children aged 12–14 years with DMFT ≥ 5, 61.1% had tooth decay.[14] Many studies show that for athletes, where good oral health is expected, the actual results do not confirm it. A review study on the oral health of elite athletes found caries in up to 75% of them.[5]

Increased consumption of carbohydrate-rich foods may contribute to the risk of caries, tooth erosion and periodontal inflammation in athletes, especially when they also exhibit dehydration due to endurance training and poor oral hygiene habits.

The present study also provided data on hygiene habits, related to the time and frequency of tooth cleaning, and the type of toothbrush used, as well as the frequency of dental visits in both groups. Swimmers and the control group brushed their teeth twice a day (66.7% vs. 80.0%), with swimmers brushing more often before breakfast (60.0%) and the control group after breakfast (70.0%).

Before sleep, both groups cleaned their teeth similarly (93.9% vs. 100.0%). The participants – both swimmers and controls – used a manual toothbrush in most cases (86.6% vs. 100.0%). Similar responses were obtained by Mulic et al. in a study of people in Norway, actively engaged in sports, who declared brushing their teeth twice a day and making regular dental visits at intervals ranging from 6 months to 2 years.[30] In a study conducted among Finnish adolescents actively engaged in sports, 95.0% brushed their teeth every day and 83.6% twice a day.[31] Different results were obtained by Khan et al. in athletes from Pakistan, who overwhelmingly (51.5%) brushed their teeth once a day – in the morning, while only 28.6% brushed their teeth in the morning and before going to bed.[4] In our study, both swimmers and the non-competitive group regularly visited the dentist twice a year, with the reason being a check-up (76.6% vs. 60.0%) and less often a toothache (33.3% vs. 0.0%). In a study by Kragt et al, 81% of Dutch athletes had their last dental visit within a year.[3] Different results were obtained by Khan et al.; the researchers reported that 37.5% of athletes had never been to the dentist, with only 5.8% making regular visits (every 6–8 months).[4] In most cases (50.5%), the reason was a toothache. The lack of regular visits, or even the lack of visits, translated into a high prevalence of dental caries in the subjects, which was 63.5%, and DMFT was equal to 2.7. The higher prevalence of dental caries, associated with the lack of good hygiene habits, along with the lack of check-up visits, could be due to the fact that the study was conducted in a developing country.[4] In their cross-sectional study, Needleman et al. noted that continental location was significantly associated with dental caries.[2]

The data on dietary habits showed in both groups, except for one person, the lack of adherence to a specific diet. Similar results were presented by D'Ercole et al., studying young swimmers.[29] To achieve peak performance, athletes often consume high-carbohydrate foods and energy drinks. The amount of sugary snacks and beverages consumed by competitive swimmers differed from that in the control group. Also, swimmers consumed sweet drinks significantly more often as compared to the control group. Similar results regarding the more frequent consumption of sweet snacks and drinks by active athletes as compared to the control group were obtained by Anttonen et al.[31] In our study, the subjects did not consume beverages during training. In a study by Frese et al., conducted among athletes involved in running, biking and swimming, 45.7% consumed sports drinks, 51.4% consumed water and 2.9% did not drink during exercise.[7]

Rinsing the mouth with water or a neutral beverage after exposure to carbohydrates or acidic sports nutrition products can reduce carbohydrate contact time and more quickly restore oral pH levels to neutral, reducing the risk of tooth decay and erosion.[32] In our study, the post-workout subjects predominantly drank water, while a smaller proportion drank energy drinks.

Prolonged exercise can reduce the unstimulated salivary flow.[33] In the present study, salivary parameters were determined twice a day, i.e., in the morning and in the afternoon. Saliva is the fluid responsible for cleaning the teeth and oral mucosa, has a buffering capacity and affects pH in the mouth. By analyzing salivary parameters, especially the flow rate, pH and buffering capacity, as well as the growth of caries-forming bacteria, it is possible to assess the risk of caries.[16] The literature shows that if there is a reduced salivary flow rate, and decreased pH and buffering capacity, the incidence of caries increases.[13] A decreased salivary flow can be explained by an increase in the activity of the sympathetic nervous system during intense exercise, causing the constriction of blood vessels in the salivary glands, resulting in a decreased salivary volume.[24] It can also be a consequence of sweat-induced dehydration and restricted fluid intake during exercise. However, in our study, the heat produced by the bodies of swimmers was released directly to water of a temperature ranging from 16°C to 28°C. Therefore, they were not exposed to dehydration. The significant ($p < 0.05$) reduction in saliva secretion after swimming exercise in the morning and in the afternoon observed in our study is consistent with previous studies.[7,30] In contrast, different results were reported by Anttonen et al., who observed a significant increase in saliva secretion after exercise, which returned to the baseline value after 30 min.[31] Such a result was explained by the fact that the study was conducted at a temperature of 10°C, which limits the dehydration of the body.[31] Our own data, like the results obtained by Dimitriou et al. for swimmers,[34] showed saliva secretion to be higher in the afternoon than in the morning, which is related to the higher activity of the sympathetic system in the morning than at other times of day.

In our study, an increase in salivary pH was observed after swimming training. It is unanimously confirmed by data from other authors.[7,23] Different results were reported by Tanabe-Ikegawa et al., who observed a decrease in pH following cycling exercise, which would support the rationale that as saliva secretion decreases, pH also decreases, and this can be explained by the fact that changes in salivary pH are related to the blood levels of CO_2.[35] The level of CO_2 in blood increases with athletic performance, which consequently causes a high concentration of CO_2 to pass from blood to saliva, thus causing a decrease in salivary pH. Our own data are different, which is probably related to the training environment; swimmers are in an aquatic environment all the time and feel less exhaustion during exercise as compared to training in a dry environment.

The buffering capacity of saliva helps maintain a normal acid–base balance in the oral cavity. A high concentration of acids adversely affects the condition of the teeth in terms of dental caries. The buffering capacity is responsible for maintaining proper saliva homeostasis. In our study, an increase in the buffering capacity was observed after training in the morning in contrast to afternoon

training, where it was found to decrease. A decrease in the buffering capacity was also observed by Frese et al., who studied the effects of endurance training on oral health, with reference to tooth decay, among other factors.[7]

Moreover, an essential factor in caries prevention is the remineralization of the initial carious lesions. The protective effect on the hard tissues of the tooth is related to the presence of the Ca and phosphate ions in saliva, which are crucial for the remineralization of tooth enamel.[36] Physical exercise of varying intensity and duration can alter the concentration of these ions in saliva.[20] Our study showed a significant increase in the salivary Ca concentration after training in the morning, as well as in the afternoon, with respect to the baseline levels. This data differs from the results of Chicharro et al., who noted a slight decrease in the mean values of mixed saliva Ca levels after maximal cycloergometer exercise.[37] In contrast, our own results are consistent with those of Bretz and Carrilho, who noted a significant increase in Ca after a swim session.[38]

Conclusions

The oral health status in athletes should be considered a priority during sports participation. The prevention and promotion of oral health through greater awareness of the impact of oral hygiene and diet on the overall health of the body should become an important part of the care of athletes. Swimmers consumed carbohydrate-containing products more often as compared to the control group. Greater consumption of cariogenic products could translate into a higher number of decayed teeth in swimmers as compared to the control group. Caries prevalence in swimmers was higher than in the control group. After the workout session, both in the morning and in the afternoon, there was a significant decrease in V and a significant increase in Ca in swimmers. Comparing swimmers before training with the control group in the morning, significantly higher V and lower pH were observed in swimmers. It is necessary to raise awareness about the impact of oral health on sports performance.

Ethics approval and consent to participate

The study protocol was approved by the Bioethics Committee at Wroclaw Medical University, Poland (approval No. KB-327/2009). All examinations were conducted in accordance with the relevant guidelines and regulations, and all the recruited participants provided written informed parental consent.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Verhulst M. Why oral health should be considered a priority in athletes. 2021. https://www.drbicuspid.com/dental-specialties/smile-design/sports-dentistry/article/15378293/why-oral-health-should-be-considered-a-priority-in-athletes. Accessed February 1, 2024.
2. Needleman I, Ashley P, Petrie A, et al. Oral health and impact on performance of athletes participating in the London 2012 Olympic Games: A cross sectional study. *Br J Sports Med*. 2013;47(16):1054–1058. doi:10.1136/bjsports-2013-092891
3. Kragt L, Moen MH, Van Den Hoogenband CR, Wolvius EB. Oral health among Dutch elite athletes prior to Rio 2016. *Phys Sportsmed*. 2019;47(2):182–188. doi:10.1080/00913847.2018.1546105
4. Khan K, Qadir A, Trakman G, et al. Sports and energy drink consumption, oral health problems and performance impact among elite athletes. *Nutrients*. 2022;14(23):5089. doi:10.3390/nu14235089
5. Ashley P, Di Iorio A, Cole E, Tanday A, Needleman I. Oral health of elite athletes and association with performance: A systematic review. *Br J Sports Med*. 2015;49(1):14–19. doi:10.1136/bjsports-2014-093617
6. Kaczmarek W. The status of mineralized dental tissues in young competitive swimmers [in Polish]. *Ann Acad Med Stetin*. 2010;56(3):81–86. PMID:22053629.
7. Frese C, Frese F, Kuhlmann S, et al. Effect of endurance training on dental erosion, caries, and saliva. *Scand J Med Sci Sports*. 2015;25(3):e319–e326. doi:10.1111/sms.12266
8. Broadbent JM, Thomson WM. For debate: Problems with the DMF index pertinent to dental caries data analysis. *Community Dent Oral Epidemiol*. 2005;33(6):400–409. doi:10.1111/j.1600-0528.2005.00259.x
9. Roland E, Gueguen G, Longis MJ, Boiselle J. Validation of the reproducibility of the DMF index used in bucco-dental epidemiology and evaluation of its 2 clinical forms [in French]. *World Health Stat Q*. 1994;47(2):44–61. PMID:8073791.
10. Marthaler TM. Changes in dental caries 1953–2003. *Caries Res*. 2004;38(3):173–181. doi:10.1159/000077752
11. Nadanovsky P, Sheiham A. Relative contribution of dental services to the changes in caries levels of 12-year-old children in 18 industrialized countries in the 1970s and early 1980s. *Community Dent Oral Epidemiol*. 1995;23(6):331–339. doi:10.1111/j.1600-0528.1995.tb00258.x
12. Moradi G, Bolbanabad AM, Moinafshar A, Adabi H, Sharafi M, Zareie B. Evaluation of oral health status based on the decayed, missing and filled teeth (DMFT) index. *Iran J Public Health*. 2019;48(11):2050–2057. doi:10.18502/ijph.v48i11.3524
13. Hegde MN, Attavar SH, Shetty N, Hegde ND, Hegde NN. Saliva as a biomarker for dental caries: A systematic review. *J Conserv Dent*. 2019;22(1):2–6. doi:10.4103/JCD.JCD_531_18
14. González-Aragón Pineda AE, Pérez AG, García-Godoy F. Salivary parameters and oral health status amongst adolescents in Mexico. *BMC Oral Health*. 2020;20(1):190. doi:10.1186/s12903-020-01182-8
15. Harper Mallone LF, Boyd LD, Stegeman C. Practice paper of the Academy of Nutrition and Dietetics abstract: Oral health and nutrition. *J Acad Nutr Diet*. 2014;114(6):958. doi:10.1016/j.jand.2014.04.004
16. Tripodi D, Cosi A, Fulco D, D'Ercole S. The impact of sport training on oral health in athletes. *Dent J (Basel)*. 2021;9(5):51–62. doi:10.3390/dj9050051

17. Dawes C. Rhythms in salivary flow rate and composition. *Int J Chronobiol*. 1974;2(3):253–279. PMID:4613672.
18. Piątowska D, ed. *Kariologia Współczesna. Postępowanie Kliniczne*. Otwock, Poland: Med Tour Press International; 2009:96.
19. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K, Punyadeera C. Diagnostic potential of saliva: Current state and future application. *Clin Chem*. 2011;57(5):675–687. doi:10.1373/clinchem.2010.153767
20. Soares Nunes LA, de Macedo DV. Saliva as a diagnostic fluid in sports medicine: Potential and limitations. *J Bras Patol Med Lab*. 2013;49(4):247–255. doi:10.1590/S1676-24442013000400003
21. Soares Nunes LA, Mussavira S, Bindhu OM. Clinical and diagnostic utility of saliva as a non-invasive diagnostic fluid: A systematic review. *Biochem Med (Zagreb)*. 2015;25(2):177–192. doi:10.11613/BM.2015.018
22. Ligtenberg AJ, Brand HS, van den Keijbus PA, Veerman EC. The effect of physical exercise on salivary secretion of MUC5B, amylase and lysozyme. *Arch Oral Biol*. 2015;60(11):1639–1644. doi:10.1016/j.archoralbio.2015.07.012
23. Leicht CA, Goosey-Tolfrey VL, Bishop NC. Exercise intensity and its impact on relationships between salivary immunoglobulin A, saliva flow rate and plasma cortisol concentration. *Eur J Appl. Physiol*. 2018;118(6):1179–1187. doi:10.1007/s00421-018-3847-6
24. Ljungberg G, Ericson T, Ekblom B, Birkhed D. Saliva and marathon running. *Scand J Med Sci Sports*. 1997;7(4):214–219. doi:10.1111/j.1600-0838.1997.tb00142.x
25. Walsh NP, Laing SJ, Oliver SJ, Montague JC, Walters R, Bilzon JL. Saliva parameters as potential indices of hydration status during acute dehydration. *Med Sci Sports Exerc*. 2004;36(9):1535–1542. doi:10.1249/01.mss.0000139797.26760.06
26. Kubala E, Strzelecka P, Grzegocka M, et al. A review of selected studies that determine the physical and chemical properties of saliva in the field of dental treatment. *Biomed Res Int*. 2018;2018:6572381. doi:10.1155/2018/6572381
27. World Health Organization (WHO). Oral health surveys: Basic methods – 5th edition. Geneva, Switzerland: World Health Organization; 2013. https://www.who.int/publications/i/item/9789241548649. Accessed February 1, 2024.
28. Gallagher J, Ashley P, Petrie A, Needleman I. Oral health and performance impacts in elite and professional athletes. *Community Dent Oral Epidemiol*. 2018;46(6):563–568. doi:10.1111/cdoe.12392
29. D'Ercole S, Tieri M, Martinelli D, Tripodi D. The effect of swimming on oral health status: Competitive versus non-competitive athletes. *J Appl Oral Sci*. 2016;23(2):107–113. doi:10.1590/1678-775720150324
30. Mulic A, Tveit AB, Songe D, Sivertsen H, Skaare AB. Dental erosive wear and salivary flow rate in physically active young adults. *BMC Oral Health*. 2012;12:8. doi:10.1186/1472-6831-12-8
31. Anttonen V, Kemppainen A, Niinimaa A, Pesonen P, Tjäderhane L, Jaana L. Dietary and oral hygiene habits of active athlets and adolescents attending ordinary junior high school. *Int J Paediatr Dent*. 2014;24(5):358–366. doi:10.1111/ipd.12078
32. Broad EM, Rye LA. Do current sports nutrition guidelines conflict with good oral health? *Gen Dent*. 2015;63(6):18–23. PMID:26545270.
33. de Sant'Anna GR, Lorenzetti Simionato MR, Stama Suzuki ME. Sport dentistry: Buccal and salivary profile of a female soccer team. *Quintessence Int*. 2004;35(8):649–652. PMID:15366530.
34. Dimitriou L, Sharp NC, Doherty M. Circadian effects on the acute responses of salivary cortisol and IgA in well trained swimmers. *Br J Sports Med*. 2002;36(4):260–264. doi:10.1136/bjsm.36.4.260
35. Tanabe-Ikegawa M, Takahashi T, Churei H, Mitsuyama A, Ueno T. Interactive effect of rehydration with diluted sports drink and water gargling on salivary flow, pH, and buffering capacity during ergometer exercise in young adult volunteers. *J Oral Sci*. 2018;60(2):269–277. doi:10.2334/josnusd.17-0183
36. de Sousa Né YG, Lima WF, Santos Mendes PF, et al. Dental caries and salivary oxidative stress: Global scientific research landscape. *Antioxidants (Basel)*. 2023;12(2):330. doi:10.3390/antiox12020330
37. Chicharro JL, Serrano V, Ureña R, et al. Trace elements and electrolytes in human resting mixed saliva after exercise. *Br J Sports Med*. 1999;33(3):204–207. doi:10.1136/bjsm.33.3.204
38. Bretz WA, Carrilho MR. Salivary parameters of competitive swimmers at gas-chlorinated swimming-pools. *J Sports Sci Med*. 2013;12(1):207–208. PMID:23536742.

Original paper

Treatment of maxillary edentulism with weldable wing spindles and allogenic bone for sinus lift: A retrospective study

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Abstract

Background. The technique described in this study has been used by our group for approx. 20 years. It involves fabricating a provisional or definitive prosthesis over a metal structure made of several wing abutments that can be intraorally welded to connect the adjacent implants.

Objectives. The aim of this retrospective observational study was to evaluate the effectiveness of and the complications associated with the use of intraorally welded wing abutments in patients with edentulous maxilla undergoing sinus floor augmentation with frozen homologous bone.

Material and methods. Data from adult patients diagnosed with edentulism in the posterior maxilla were retrospectively analyzed. All patients underwent sinus augmentation with homologous bone and were rehabilitated for 5–6 months after surgery, using wing spindles. The primary outcome of the study was to evaluate the prosthetic success, while the secondary outcomes included the assessment of the implant success and the incidence of complications.

Results. Data analysis included 35 patients, corresponding to 220 implants. At the last follow-up, a 100% prosthetic success and a 96.36% implant survival rate were obtained. A total of 8 patients (22.86%, corresponding to 8.64% of total implants) experienced complications, such as radiographic radiolucency, peri-implantitis and implant mobility.

Conclusions. The results of this retrospective study suggest that patients with edentulous maxilla undergoing sinus floor augmentation with frozen homologous bone might benefit from the use of intraorally welded wing abutments.

Keywords: sinus augmentation, welded wing abutment, edentulous maxilla

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Highlights

- In 35 edentulous maxilla patients treated with sinus augmentation using frozen allogenic bone, intraorally welded wing abutments achieved a 100% prosthetic success at the last follow-up.
- Across 220 implants, the implant survival rate was ~96%, with the mean marginal bone loss (MBL) ~0.89 mm.
- Complications were infrequent (~9% of total implants) and mainly involved implants in allogenic bone (radiolucency, peri-implantitis, mobility).

Introduction

The rehabilitation of edentulous patients is usually managed by using prostheses supported by multiple implants,[1] which can be splinted with a titanium bar to achieve better performance.[2] This approach allows to increase the mechanical stability of implants,[3,4] ultimately reducing the risk of implant and prosthetic failure.[5]

Since the placement and modeling of the bar can be challenging, the pair-by-pair splinting of the adjacent implants can be a valuable alternative facilitating the whole procedure. This can be done resorting to wing abutments, which are welded intraorally. Their application has already proven to be a viable approach for the rehabilitation of both partially and totally edentulous patients, even in immediate loading scenarios.[6,7]

In principle, the mechanical stability provided by wing spindles could be advantageous also for patients needing sinus floor augmentation, a commonly used technique when the residual bone height of the posterior maxilla is less than 4 mm.[8] This approach consists in opening a lateral window to access the sinus cavity, displacing the sinus membrane, and finally filling the cavity with a bone substitute.[9–11]

Table 1. Study inclusion and exclusion criteria

Inclusion criteria
– age between 18 and 90 years – diagnosis of edentulism in the posterior maxilla – no contraindications for osteo-reconstructive surgery – undergoing implant insertion and the sinus lift procedure at the same time
Exclusion criteria
– coagulation or white blood cells pathologies – non-controlled cardiocirculatory pathologies – immunodeficient patients – non-controlled metabolic pathologies – head-to-neck radiotherapy within the last 24 months – intravenous bisphosphonate – active smoking (>10 cigarettes/day) – sinus affections and deformity – respiratory pathologies – previous sinus augmentation surgery – sensibility to anesthetic drugs – local infections – non-controlled periodontitis – undergoing implant insertion and the sinus lift procedure at different times

Having osteoinductive, osteoconductive and osteogenic properties, as well as no risk of immunological rejection or disease transmission, autologous bone might be regarded as the most suitable bone graft. However, given the invasiveness and potential complications associated with the sampling of autologous bone,[12] heterologous and homologous grafts represent valuable alternatives[13–16] with proven osteoconductive capabilities.[17] While requiring some processing, such as freezing, lyophilization and demineralization, allogenic bone has a long history of use for bone augmentation and has proven to be comparable to autologous bone.[13,18–20]

By combining the aforementioned techniques, the aim of this study was to evaluate the effectiveness of and the complications associated with the use of wing spindles in patients with edentulous maxilla undergoing sinus floor augmentation with frozen allogenic bone.

Material and methods

Study design and patients' characteristics

This was a retrospective, observational, monocentric study evaluating the clinical records of patients treated at the Authors' clinic between 1997 and 2019. The inclusion and exclusion criteria are summarized in Table 1.

The study protocol was assessed and approved by the relevant ethics committee (Comitato Etico per le Sperimentazioni Cliniche (CESC) della Provincia di Vicenza, Italy; approval No. 66/22). All procedures were performed in accordance with the good clinical practice (GCP) and the Declaration of Helsinki.

Allogenic bone processing

Allogenic bone was provided by Fondazione Banca dei Tessuti del Veneto (FBTV; Treviso, Italy), an institution accredited by the Italian National Transplant Centre for the retrieval, processing, storage, and distribution of human tissues for transplantation. Tissue donors were selected according to the Italian directives, which stipulate donor anamnesis and blood testing for human immunodeficiency virus (HIV)-1 and HIV-2 antibodies (Ab),

human T-cell leukemia/lymphoma virus (HTLV)-1 and HTLV-2 Ab, hepatitis B virus (HBV) surface antigen and anti-core Ab, hepatitis C virus (HCV) Ab, and syphilis. Screening also included cytomegalovirus Ab, and nucleic acid amplification tests (NAT) for HIV, HBV and HCV. Tissues were retrieved within 24 h from cardiac arrest and processed under class A laminar flow hoods. Bone blocks were obtained from the iliac crests and decontaminated in a validated antibiotic.[21,22] Afterward, the bone blocks were stored at −80°C. Several microbiological tests were carried out throughout the processes, and only uncontaminated tissues with excellent morphology were distributed for clinical implantation.

Description of the surgical procedure and implant placement

After clinical examinations and radiographic asessments using intraoral radiographs and cone-beam computed tomography (CBCT), the diameters, lengths and positions of the implants were pre-planned on the CBCT scans, and a surgical guide was manufactured.

The patients were prescribed antibiotic prophylaxis (2 g amoxicillin/clavulanic acid (Augmentin); GlaxoSmithKline, Verona, Italy) 1 h before surgery and every 12 h for 8–10 days after surgery. The surgical procedure was carried out under local anesthesia with articaine hydrochloride (40 mg/mL) and epinephrine (1:100,000) on the vestibular and palatal sides. Mucosae were cleaned with iodine. The gingiva was incised paracrestally and a full-thickness flap was raised to create an access window on the lateral wall of the maxillary sinus. Access to the sinus was obtained using a round tungsten bur. Then, the sinus membrane was carefully elevated and the window was gently pushed inside the cavity. The procedure was performed under irrigation with sterile saline.

Based on the CBCT scans, the allogenic bone block was shaped with a bur to fit the cavity through a process of trial and error. Once placed in the sinus, the block was simultaneously pressed against the sinus base with a spatula and drilled through the bone ridge to accommodate the implant. Then, the bone ridge and the bone block were further drilled following the drill sequence recommended by the implant manufacturer, while keeping the block pressed against the sinus base with a spatula. Afterward, the implants were placed using the guide, sealed with a cover screw, and the gingiva was sutured.

When required, implants were placed in native bone to rehabilitate mesially located edentulous gaps, whereas posterior implants were stabilized using sinus grafting. The implants were 10-11.5-13-15 mm long and 3.25 or 4 mm wide. All implants were of the same brand and from the same manufacturer (BTK®; Biotec, Povolaro, Italy), featuring a tapered design with a sand-blasted, double-etched surface.

Prosthetic rehabilitation

All patients were rehabilitated for 5–6 months after surgery, using wing spindles (Wings®; T.A.B., Borso del Grappa, Italy), which are available in different heights (1.7, 2.7 and 4.5 mm). The wing spindles were connected to the implants through 20-mm-long screws and their lateral 'wings' were cut at the desired length to partially overlap those of the adjacent implants. Finally, the wing spindles were welded intraorally.

The resulting metal structure constituted the internal reinforcement of the prosthesis. This was fabricated by first taking an alginate impression of the sub-structure to create a cast. Based on this metal structure, the technician prepared the definitive prosthesis, using a composite resin. Lastly, using the same composite resin, the screw holes were filled.

More details on the pair-by-pair splinting technique can be found elsewhere.[6,7]

Control visits

Patients underwent ortopantomography (OPG) and sinus augmentation at baseline (T0). A second OPG was taken 5–6 months later, when the prosthesis was delivered (T1). Further OPGs were scheduled at variable intervals based on individual needs; regardless of these additional assessments, an OPG was obtained for each patient at 12 months from baseline (T2). At each follow-up visit, the prosthesis was unscrewed to facilitate proper hygiene for the patient. This also enabled the assessment of implant osseointegration, and the detection of any signs of peri-implantitis or mucositis.

Study endpoints

The primary endpoint of the study was to evaluate the prosthetic success. A prosthesis was deemed successful if none of the following events occurred: prosthesis unscrewing, chipping or fracture; screw loosening or fracture; or welding point fracture.

The secondary endpoint consisted in the assessment of implant survival with respect to the marginal bone loss (MBL), as well as the implant success evaluated according to the Albrektsson and Zarb criteria. These criteria included: the absence of persistent pain, dysesthesia or paresthesia in the implant area; the absence of peri-implant infection with or without suppuration; the absence of perceptible implant mobility; and the absence of more than 1.5 mm or peri-implant bone resorption during the first year of loading or 0.2 mm/year of resorption during the following years. The implants were considered successful when all the abovementioned conditions were met.

In addition, the incidence of complications (the loss of implant, peri-implantitis, pain, edema, or other adverse events) was recorded.

MBL measurement

For all the included records, the intraoral radiographs were digitally scanned, converted to 600 dpi resolution TIFF images, stored on a personal computer, and analyzed using image analysis software (ImageJ, National Institutes of Health (NIH), Bethesda, USA; https://imagej.net/ij) to measure the peri-implant MBL. The process was as follows: after loading each image, the software was calibrated using the known implant diameter at the most coronal portion of the implant neck; then, the distance from the implant–abutment interface to the most apical point of crestal bone in intimate contact with the implant was measured to the nearest 0.01 mm on both the mesial and distal sides. These 2 measurements were averaged to obtain a single peri-implant MBL value. The MBL for each implant at the final follow-up visit was calculated by subtracting the baseline peri-implant bone level (measured at implant insertion) from the bone level at the follow-up time point.

Bias

To mitigate potential bias from the fact that the patients were treated exclusively by one of the authors (SD), the selection of clinical records and data extraction were performed by other authors (FM, NZ). Additionally, to further address any possible sources of bias, an independent biostatistician conducted the statistical analysis.

Statistical analysis

Since the study aimed to investigate the prosthetic success using descriptive statistics, no sample size calculation was performed. Thus, the study population size corresponds to the number of records that met the inclusion criteria.

Absolute and relative frequencies were used to describe categorical variables (the patients' age, sex, the prosthetic success, the implant success, and complications), while mean and standard deviation (M ±SD), and median and interquartile range (Me (IQR)) were used to report continuous variables. Normality was checked by means of the Shapiro–Wilk test. All analyses were performed in Origin 2022 (OriginLab, Northampton, USA).

Results

The study included 35 patients (N = 35), 19 males (54%) and 16 females (46%), with a mean age at surgery of 55.1 ±7.1 years (range: 42–67 years). The total number of implants was 220, of which 60 were placed in native bone and 160 in allogenic bone. Half of the implants (n = 110) were placed in the right and left maxilla, and the number of implants in each patient was distributed as follows: 8 patients (22.86%) received 7 implants; 6 patients (17.14%) received 8 implants; 6 patients (17.14%) received 6 implants; 5 patients (14.29%) received 3 implants; 3 patients (8.57%) received 4 implants; 2 patients (5.71%) received 10 implants; 2 patients (5.71%) received 5 implants; 1 patient (2.86%) received 12 implants; 1 patient (2.86%) received 9 implants; and 1 patient (2.86%) received 2 implants. The mean follow-up period was 122.2 ±68.0 months (range: 25–296 months) after implant insertion. The patients' characteristics and rehabilitation details are summarized in Table 2.

The prosthetic success was obtained in all patients (N = 35; 100%,), with an implant survival rate of 96.36% (n = 212). According to the Albrektsson and Zarb criteria, 197 implants (89.55%) were successful. The average MBL was 0.89 ±0.54 mm (range: 0.12–2.79 mm).

Complications were observed in 8 patients (22.86%), corresponding to 8.64% (n = 19) of delivered implants. All complications except one occurred in the implants placed in allogenic bone. Specifically, non-early osseointegration involved 3 implants (1.36%), radiographic radiolucency – 8 implants (3.64%), peri-implantitis – 4 implants (1.82%), and implant mobility – 1 implant (0.45%). Moreover, 1 implant delivered to a non-smoking patient was lost after 57 months due to peri-implantitis. Another patient with previous chronic periodontitis, recurrent bleeding and a significant muscular strength/chewing force, lost 2 implants after 115 months. Figures 1 and 2 show 2 different cases which were successfully rehabilitated with the proposed technique.

Discussion

Taking into account a 100% prosthetic success and an implant survival rate of 96.36%, the results of this retrospective study suggest that patients with edentulous maxilla undergoing sinus floor augmentation with frozen allogenic bone might benefit from the use of wing abutments.

To the best of the authors' knowledge, the implant survival rate obtained in this study appears to be similar to, or even higher than those observed in the scarce literature reporting on similar procedures. Indeed, Avvanzo et al., who retrospectively evaluated the survival rate of intraorally welded implants delivered in patients undergoing either sinus augmentation or crest splitting, found that the 1-year survival rate in the group undergoing sinus lift was 83.4%.[23] While sample sizes are quite different (21 vs. 220 implants), the higher survival rate observed in the present study (96.36%) might be also ascribed to the welding of wing abutments rather than titanium bars as one of the existing differences between the compared studies.

More in line with the herein presented results, Rizzo et al. reported a 97.7% survival rate of the implants placed immediately after transcrestal sinus augmentation

Table 2. Patients' characteristics and rehabilitation details

Patient ID	Age [years]	Gender	Follow-up period [months]	Implants in native bone (*n*)	Implants in allogenic bone (*n*)	Complications
N1	55	M	25	3	5	–
N2	63	M	43	3	5	–
N3	63	M	49	3	4	–
N4	59	M	44	0	6	non-early osseointegration
N5	52	F	72	1	2	–
N6	51	M	80	3	4	–
N7	53	F	103	1	5	radiographic radiolucency
N8	66	M	103	2	6	–
N9	58	F	139	2	6	radiographic radiolucency, peri-implantitis, implant loss
N10	50	M	140	4	5	–
N11	59	F	67	2	4	–
N12	45	M	111	0	3	–
N13	58	F	122	3	4	–
N14	60	F	116	1	4	–
N15	56	F	110	3	4	–
N16	51	M	105	1	5	radiographic radiolucency
N17	53	F	129	1	6	non-early osseointegration, radiographic radiolucency
N18	60	F	95	0	6	radiographic radiolucency, peri-implantitis, implant loss
N19	54	M	118	5	5	–
N20	67	M	129	0	8	–
N21	42	F	296	0	7	–
N22	47	M	265	2	6	–
N23	63	M	233	0	5	–
N24	52	F	242	1	6	radiographic radiolucency, peri-implantitis, implant mobility
N25	48	M	261	6	6	–
N26	53	M	228	0	6	–
N27	45	M	53	2	1	non-early osseointegration
N28	48	F	78	0	2	–
N29	67	F	110	5	5	–
N30	60	F	89	2	2	–
N31	61	F	71	1	3	–
N32	54	M	131	1	3	–
N33	66	M	90	0	3	–
N34	43	F	129	1	6	–
N35	47	M	102	1	2	–

M – male; F – female.

with fresh-frozen allogenic bone blocks.[24] Similarly, Kim et al. obtained a 97.06% failure-free survival rate at 1 year after implant placing and sinus augmentation.[25]

Nevertheless, it must be said that the implant success rate observed in the present study (89.55%) seems to be lower than that achieved when intraorally welded

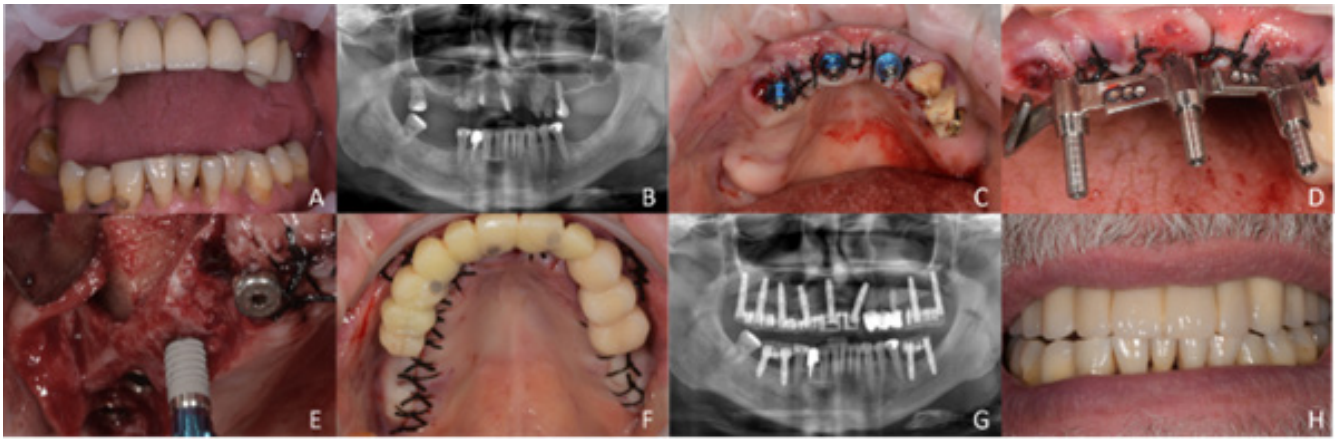

Fig. 1. Case 1

A – full mouth picture; B – ortopantomograph (OPG) before surgery; C – delivery of new implants in native bone after the removal of the former prosthesis; D – rehabilitation using wing spindles; E – implant placement in the sinus filled with the modeled allogenic bone block in the posterior maxilla; F – rehabilitation with the definitive prosthesis made of a composite resin; G – OPG at the end of the procedure; H – full mouth picture at the end of the procedure.

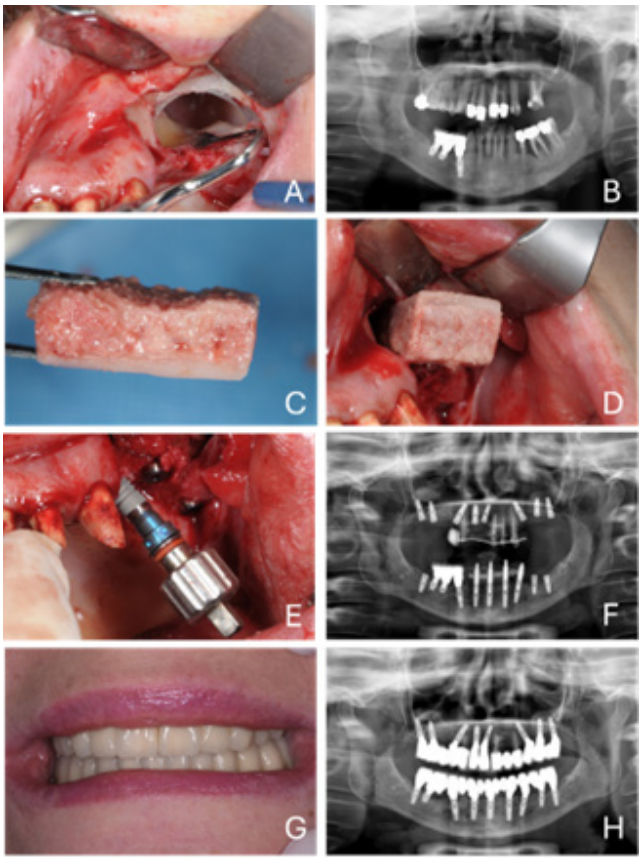

Fig. 2. Case 2

A – opening of the lateral window to access the sinus cavity; B – OPG before surgery; C – picture of the allogenic bone block prior to implantation; D – filling of the sinus with the allogenic bone block; E – implant placement in the sinus filled with the modeled allogenic bone block; F – OPG during the rehabilitation process; G – full mouth picture at the end of the procedure; H – OPG at the end of the procedure.

titanium bars are used in fully or partially edentulous patients not undergoing sinus augmentation, and in arches area other than the posterior maxilla. Indeed, when investigating such clinical scenarios, Degidi et al. achieved very high implant success rates, which were close to 100% both at 1 and 2 years of follow-up.[4,26]

The combination of the herein proposed techniques also proved to be safe, as most of the recorded complications were related to radiographic radiolucency, which is generally considered an artifact caused by beam hardening.[27] Moreover, soft-tissue complications, such as peri-implantitis, occurred only in patients with a previous clinical history of recurrent periodontitis.

The results of this study align with previous retrospective analyses evaluating the success and complication rates of the pair-by-pair splinting technique for rehabilitating completely edentulous patients.[6] In those patients, the prosthetic success rate was 100%, and the implant survival rate was 97.2%.[6] This study not only supports the viability of the technique, but also extends its application to patients requiring sinus floor augmentation. Notably, the technique is straightforward and quick to perform, as wing abutment extensions come in different angles, allowing the metal frame to be shaped according to each patient's specific anatomy. Additionally, this approach is more cost-effective as compared to other rehabilitation procedures, making it a viable option with acceptable esthetic and functional outcomes for those unable to afford more expensive treatment.

Besides its retrospective nature, the main limitations of the present study include the heterogeneous dimensions and number of implants per patient, as well as the evaluation of a follow-up period with a wide range. Prospective and/or comparative studies will be necessary to assess the effectiveness and safety of the proposed approach without relevant bias.

Conclusions

Within its limitations, the results of the present study suggest that the rehabilitation of patients with edentulous maxilla requiring sinus floor augmentation can be safely and successfully performed by combining the use of frozen allogenic bone with intraorally welded wing abutments.

Ethics approval and consent to participate

The study protocol was assessed and approved by the relevant ethics committee (Comitato Etico per le Sperimentazioni Cliniche (CESC) della Provincia di Vicenza, Italy; approval No. 66/22). All procedures were performed in accordance with the good clinical practice (GCP) and the Declaration of Helsinki.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Lee DJ, Saponaro PC. Management of edentulous patients. *Dent Clin North Am*. 2019;63(2):249–261. doi:10.1016/j.cden.2018.11.006
2. Dal Carlo L, Pasqualini ME, Mondani PM, Rossi F, Moglioni E, Shulman M. Mondani intraoral welding: Historical process and main practical applications. *J Biol Regul Homeost Agents*. 2017;31(2 Suppl 1):233–239. PMID:28691478.
3. Degidi M, Nardi D, Piattelli A. Immediate loading of the edentulous maxilla with a definitive restoration supported by an intraorally welded titanium bar and tilted implants. *Int J Oral Maxillofac Implants*. 2010;25(6):1175–1182. PMID:21197495.
4. Degidi M, Nardi D, Piattelli A. Immediate rehabilitation of the edentulous mandible with a definitive prosthesis supported by an intraorally welded titanium bar. *Int J Oral Maxillofac Implants*. 2009;24(2):342–347. PMID:19492651.
5. Pasqualini ME, Lauritano D, Rossi F, et al. Rehabilitations with immediate loading of one-piece implants stabilized with intraoral welding. *J Biol Regul Homeost Agents*. 2018;32(2 Suppl 1):19–26. PMID:29460514.
6. Zuffellato N, Massariol M, Gandini A, Dovigo S. Intraorally welded wing abutments supporting full-arch prostheses: A retrospective clinical study. *Dent Med Probl*. 2022;59(3):333–341. doi:10.17219/dmp/149470
7. Dovigo S, Massariol M, Gandini A, Zuffellato N. Instantaneous dental implant loading technique by fixed dentures: A retrospective cohort study. *Dent Med Probl*. 2023;60(3):375–383. doi:10.17219/dmp/154981
8. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. *J Oral Surg*. 1980;38(8):613–616. PMID:6993637.
9. Sousa Rocha LR, Aloise AC, De Mello Oliveira R, Teixeira ML, Pelegrine AA, Scavone Macedo LG. Long-term retrospective study of implants placed after sinus floor augmentation with fresh-frozen homologous block. *Contemp Clin Dent*. 2017;8(2):248–252. doi:10.4103/ccd.ccd_63_17
10. McAllister BS, Haghighat K. Bone augmentation techniques. *J Periodontol*. 2007;78(3):377–396. doi:10.1902/jop.2007.060048
11. Albanese M, Ricciardi G, Luciano U, et al. Alveolar splitting with Piezosurgery®, bone bank grafts and NobelActive implants as an alternative to major bone grafting for maxillary reconstruction. *Minerva Stomatol*. 2019;68(1):3–10. doi:10.23736/S0026-4970.17.04006-7
12. Fowler BL, Dall BE, Rowe DE. Complications associated with harvesting autogenous iliac bone graft. *Am J Orthop (Belle Mead NJ)*. 1995;24(12):895–903. PMID:8776079.
13. Danesh-Sani SA, Engebretson SP, Janal MN. Histomorphometric results of different grafting materials and effect of healing time on bone maturation after sinus floor augmentation: A systematic review and meta-analysis. *J Periodontal Res*. 2017;52(3):301–312. doi:10.1111/jre.12402
14. Schwartz Z, Mellonig JT, Carnes DL Jr., et al. Ability of commercial demineralized freeze-dried bone allograft to induce new bone formation. *J Periodontol*. 1996;67(9):918–926. doi:10.1902/jop.1996.67.9.918
15. Bianchini MA, Buttendorf AR, Benfatti CA, Bez LV, Ferreira CF, De Andrade RF. The use of freeze-dried bone allograft as an alternative to autogenous bone graft in the atrophic maxilla: A 3-year clinical follow-up. *Int J Periodontics Restorative Dent*. 2009;29(6):643–647. PMID:20072742.
16. Kao ST, Scott DD. A review of bone substitutes. *Oral Maxillofac Surg Clin North Am*. 2007;19(4):513–521. doi:10.1016/j.coms.2007.06.002
17. Valentini P, Artzi Z. Sinus augmentation procedure via the lateral window technique – reducing invasiveness and preventing complications: A narrative review. *Periodontol 2000*. 2023;91(1):167–181. doi:10.1111/prd.12443
18. Viscioni A, Franco M, Rigo L, Guidi R, Spinelli G, Carinci F. Retrospective study of standard-diameter implants inserted into allografts. *J Oral Maxillofac Surg*. 2009;67(2):387–393. doi:10.1016/j.joms.2008.06.099
19. Viscioni A, Franco M, Paolin A, et al. Effectiveness of fresh frozen and cryopreserved homologue iliac crest grafts used in sinus lifting: A comparative study. *Cell Tissue Bank*. 2011;12(4):263–271. doi:10.1007/s10561-010-9192-6
20. Procopio O, Trojan D, Frigo AC, Paolin A. Use of homologous bone for alveolar crest reconstruction in 483 patients with 5 years' outcomes post implantation. *Oral Maxillofac Surg*. 2019;23(3):353–363. doi:10.1007/s10006-019-00781-2
21. Serafini A, Riello E, Trojan D, et al. Evaluation of new antibiotic cocktails against contaminating bacteria found in allograft tissues. *Cell Tissue Bank*. 2016;17(4):619–628. doi:10.1007/s10561-016-9581-6
22. Montagner G, Trojan D, Cogliati E, Manea F, Vantini A, Paolin A. Stability analysis of the antibiotic cocktail used by Treviso Tissue Bank Foundation for tissues decontamination. *Cell Tissue Bank*. 2018;19(4):721–726. doi:10.1007/s10561-018-9725-y
23. Avvanzo P, Fabrocini LA, Ciavarella D, Avvanzo A, Lo Muzio L, De Maio RA. Use of intraoral welding to stabilize dental implants in augmented sites for immediate provisionalization: A case report. *J Oral Implantol*. 2012;38(1):33–41. doi:10.1563/AAID-JOI-D-10-00047
24. Rizzo R, Quaranta A, De Paoli M, Rappelli G, Piemontese M. Three-dimensional bone augmentation and immediate implant placement via transcrestal sinus lift: Five-year clinical outcomes. *Int J Periodontics Restorative Dent*. 2018;38(1):95–101. doi:10.11607/prd.2733
25. Kim BJ, Kim CH, Kim JH. Immediate implant placement following tooth extraction with simultaneous lateral sinus augmentation: A retrospective clinical study after at least 1 year of loading. *Int J Implant Dent*. 2021;7(1):96. doi:10.1186/s40729-021-00377-0
26. Degidi M, Nardi D, Piattelli A. Prospective study with a 2-year follow-up on immediate implant loading in the edentulous mandible with a definitive restoration using intra-oral welding. *Clin Oral Implants Res*. 2010;21(4):379–385. doi:10.1111/j.1600-0501.2009.01865.x
27. Terrabuio BR, Carvalho CG, Peralta-Mamani M, Da Silva Santos PS, Fischer Rubira-Bullen IR, Fischer Rubira CM. Cone-beam computed tomography artifacts in the presence of dental implants and associated factors: An integrative review. *Imaging Sci Dent*. 2021;51(2):93–106. doi:10.5624/isd.20200320

Original paper

Sleep-related respiratory impairment and psychosocial stress in patients with persistent orofacial pain: A cross-sectional exploratory study

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Abstract

Background. Sleep-related breathing disorders (SRBD), including obstructive sleep apnea (OSA) and habitual snoring, can substantially impair quality of life (QoL). However, evidence regarding their impact in patients with orofacial pain (OFP) remains limited. Although disentangling the specific consequences of SRBD from the overlapping conditions is challenging, validated psychometric instruments provide a valuable means of screening for associated biopsychosocial burdens, such as depression and anxiety.

Objectives. The aim of the present study was to investigate potential associations between respiratory distress caused by SRBD, using self-report data and psychometric variables as indicators of psychosocial stress, and the overall well-being in individuals with OFP.

Material and methods. Anonymized data from 415 patients was analyzed. The participants were screened and categorized according to the presence or absence of respiratory distress. Psychometric measures were examined descriptively. Based on the responses (positive or negative) to the screening question, group differences were evaluated within subgroups matched for sex, age decade, employment status, body mass index (BMI), and typical pain intensity. Associations between the variables were subsequently analyzed using multiple regression models.

Results. Most patients were female ($n = 290$; 69.9%), aged 20–59 years ($n = 296$; 71.3%), workers ($n = 246$; 59.3%), with a normal weight ($n = 255$; 61.4%) and without any respiratory distress ($n = 324$; 78.1%). Most patients who reported respiratory distress were female ($n = 57$; 62.6%), and more than a half were 40–59 years old ($n = 49$; 53.8%). For all domains of psychosocial stress, the percentage of patients reaching a clinically relevant score was higher for the group with respiratory distress, except for the Dysmorphic Concern Questionnaire (DCQ). Significant effects of typical pain intensity were observed across all questionnaires except DCQ, the Injustice Experience Questionnaire (IEQ), the Patient Health Questionnaire-9 (PHQ-9), and the Patient Health Questionnaire for Stress (PHQ-Stress).

Conclusions. In this cross-sectional study, about 22% of the evaluated patients reported some form of SRBD. Moreover, SRBD were associated with higher psychometric scores, suggesting a close relationship between sleep-related respiratory difficulties and psychosocial impairment.

Keywords: snoring, anxiety, depression, sleep apnea, psychosocial stress

Highlights

- Sleep-related respiratory complaints were common in this persistent orofacial pain/temporomandibular disorders (OFP/TMD) sample: ~22% reported snoring and/or apnea symptoms on screening.
- Respiratory distress was consistently associated with greater psychosocial burden, with higher rates of clinically relevant scores across multiple domains, particularly anxiety, global mental health distress, perceived injustice, and pain-related disability.
- After adjusting for demographics, the employment status, the body mass index (BMI), and pain intensity, respiratory distress remained an independent predictor of worse scores with regard to GAD-7, IEQ, PHQ-4, and craniofacial pain-related disability (GCPS-H).
- Typical pain intensity and the disability status were strong contributors to adverse psychometric profiles, reinforcing the need for an integrated biopsychosocial approach to assessment and management.
- Clinical implication: Screening for symptoms of sleep-related breathing disorders (SRBD) in OFP/TMD services may help identify patients at higher psychosocial risk, and facilitate targeted referral and interdisciplinary management.

Introduction

Sleep-related breathing disorders (SRBD) are among the most common sleep disorders, constituting a major public health concern with a paramount impact on quality of life (QoL).[1] The SRBD group ranges in severity from habitual snoring, where there is no severe gas exchange abnormality nor sleep fragmentation, to obstructive sleep apnea (OSA). The latter is a condition caused by the repetitive narrowing and collapsing of the upper airway during sleep.[2,3] Obstructive sleep apnea, which results in intermittent hypoxia, hypercapnia, as well as recurrent arousals, is the most common breathing disorder with a prevalence ranging from 9% to 38% in the general population.[4,5] When only considering the adult population, this prevalence increases to 15–75%.[3] The OSA symptoms include snoring sounds, disturbed sleep, excessive daytime sleepiness, impaired neurocognitive functions, and mood disorders, all of which have a major influence on patients' overall well-being. Aside from being indicative of OSA, snoring alone is commonly referred to as primary snoring, intermittent snoring or habitual snoring.[6] Habitual snoring in the absence of OSA has a reported prevalence ranging from 10% to 60%.[7] This condition may have a strong impact on sleep quality for both snorers and their bedfellows, ultimately leading to increased daytime anxiety, risk of depression and stress.[8]

A growing body of research has demonstrated that OSA and habitual snoring are associated with cognitive, behavioral and psychosocial problems.[9] Specifically, the daytime symptoms of both conditions deserve particular attention, as they pertain to marked psychological distress and adverse psychosocial consequences.[10] Sleep and well-being are therefore interrelated.[11] Psychosocial stress refers to a state of emotional suffering that encompasses a diversity of comorbid psychological factors, such as depressive and anxiety symptoms, general stress, and sleep disturbance.[12,13] Depression and anxiety as measures of compromised well-being are commonly reported in adults with OSA.[14,15] Psychiatric symptoms have also been reported to be more frequent in the snoring population as compared to non-snorers.[16]

That being said, the consideration of biopsychosocial outcomes when assessing the impact of poor quality of sleep has evoked a great deal of interest among the scientific community over the last decades. Currently, there is a diverse set of psychometric instruments widely used for clinical care and research, such as the Hospital Anxiety and Depression Scale (HADS), the Patient Health Questionnaire (PHQ), the Pain Catastrophizing Scale (PCS), and the Graded Chronic Pain Scale (GCPS).[17] The most common factors assessed by these tools include somatic symptoms, emotional functioning, pain coping, and perceived stress.[18] Furthermore, socio-demographic variables, such as gender, age, the body mass index (BMI), the employment status, or drinking habits, should be considered when performing a psychometric assessment, as their impact on the aforementioned factors is well-documented.[19–22] With this in mind, the Web-based Instrument for Interdisciplinary Symptom Evaluation (WISE) was applied in this study. WISE is an online tool which relies on self-report data of patients.[23] Its structure combines a symptom-burden checklist with validated psychometric in-depth questionnaires serving as case-finding instruments, and it further reports pain features, gender, age, BMI, and the employment status. This approach provides single-case summary reports from a biopsychosocial perspective, assisting clinicians with a comprehensive screening for providing tailored management to patients with orofacial pain (OFP) and/or temporomandibular disorders (TMD). Such assessment is relevant from a pain management standpoint, as psychosocial factors may influence treatment outcomes not only in patients experiencing OFP and/or TMD, but many other types of chronic pain.[23] Research has shown that OFP patients report poor emotional and social functioning, and reduced QoL.[24]

On the other hand, OFP disorders, i.e., those experienced in the head, mouth and face area, are cross-sectionally related to depression, fatigue and pain-related sleep disturbance.[24,25]

The aim of the present study was to investigate potential associations between respiratory distress caused by SRBD, using self-report data and psychometric variables as indicators of psychosocial stress, and the overall well-being in individuals with OFP.

Material and methods

Subjects

This study included anonymized WISE data from 415 patients who reported to an Interdisciplinary Orofacial Pain Unit, in Zurich, Switzerland. The patients completed the WISE questionnaire prior to their first medical appointment. The spectrum of diagnoses encountered in this unit has been reported elsewhere.[26]

According to the Swiss law, the analysis of strictly anonymized data does not require the approval by an ethics committee. Only data from patients who had given consent to the use of their anonymized data for research was used. Also, the screening question "During the last 4 weeks, how much have you been bothered by any of the following problems: snoring/apnea during sleep?" was a mandatory response item in the WISE questionnaire.

WISE dataset

As mentioned above, WISE is an instrument used for interdisciplinary subject-tailored assessment that combines a symptom-burden checklist addressing diverse symptom domains according to the PHQ-15 with validated psychometric in-depth questionnaires serving as case-finding instruments. The latter are presented to the patient when their checklist scores exceed a predefined threshold value. The psychometric measures from WISE used in this study are listed below.

Brief Illness Perception Questionnaire (IPQ)[27]

The IPQ assesses cognitive and emotional representations of illness and health threat. This questionnaire has 8 questions covering different aspects of illness perception, which are rated on a 11-point numeric response scale ranging from 0 to 10. The total score ranges from 0 to 80, and no cut-off score (CO) has been reported.

Dysmorphic Concern Questionnaire (DCQ)[28]

The DCQ is a psychometric measure assessing excessive preoccupation or concern with an imagined or slight physical defect that has a significant influence on psychosocial functioning. It consists of 7 items covering different aspects of dysmorphic concern, scored on a 4-point ordinal scale (0: not at all – 3: much more than most people). The total score ranges from 0 to 21, and a CO of 9 is indicative of a possible body dysmorphic disorder.

Graded Chronic Pain Scale (GCPS), v. 2.0[29]

The GCPS is a multidimensional measure that assesses 2 dimensions of overall chronic pain severity: pain intensity; and pain-related disability. Both dimensions are scored independently, including 3 items for pain intensity and 4 items for pain-related disability. Scores are calculated for 3 subscales: pain intensity (the assessment of current, worst and average pain intensity for the last 30 days; 0: no pain – 10: pain as bad as it could be); the disability days score (the number of days being kept from daily activities by pain for the last 3 months; the number of days determines a disability score ranging from 0–3); and the interference score (interference by pain during daily, social and working activities; 0: no interference – 10: unable to carry on any activities for the last 30 days). The latter two are used to calculate the disability points score, which ranges from 0 to 6. Two separate disability scores due to pain in the head (GCPS-H) or the body (GCPS-B) were calculated. A CO of 3 was used as indicative of high disability and severe limitation.

General Anxiety Disorder-7 (GAD-7)[30]

The GAD-7 is a screening tool for general anxiety in primary care patients. Using a 4-point ordinal rating scale, 7 items covering different aspects of general anxiety are scored between 0–3 (0: not at all – 3: nearly every day). Summary scores range from 0 to 21 and are assigned to the corresponding categories: "none/minimal" (0–4); "mild" (5–9); "moderate" (10–14); or "severe" (>14). The CO has been established at 10.

Injustice Experience Questionnaire (IEQ)[31]

The IEQ assesses injustice experienced due to accidents, injuries or maltreatment, and consists of 12 items reflecting the frequency of thoughts, beliefs and emotions associated with an injury. Each item is rated on an ordinal scale ranging from 0 to 4 (0: never – 4: all the time). The total score ranges from 0 to 48, with a CO of 18 being indicative of further professional assessment.

Pain Catastrophizing Scale (PCS)[32]

The PCS assesses catastrophizing thoughts, feelings and corresponding behaviors that patients may experience. This questionnaire has 13 items rated on a 5-point ordinal scale (0: not at all – 4: all the time), and total scores

range from 0 to 52. The PCS can be categorized into 3 subscales, each one with a specific cut-off point: rumination; magnification; and helplessness. When considering the questionnaire as a whole, a CO of 30 should be considered as clinically relevant.

Patient Health Questionnaire-4 (PHQ-4)[33]

The PHQ-4 is an ultra-brief screener of both anxiety and depression in primary care patients. It consists of a 2-item depression scale (PHQ-2, which is part of PHQ-9) and a 2-item anxiety scale (GAD-2, which is part of GAD-7). The items are rated on an ordinal scale of 0–3 (0: not at all – 3: nearly every day). The maximum score can be calculated for the 2 subscales (max = 6) or for the questionnaire as a whole (max = 12). Sum scores ≥6 for the total score and ≥3 for the subscores indicate the need for further expert evaluation.

Patient Health Questionnaire-9 (PHQ-9)[34]

The PHQ-9 screens for depression and assesses its severity. As GAD-7, it correlates with functional impairment. Nine items approaching different aspects of depression are scored using the same scale as the PHQ-4. Total scores range from 0 to 27 and are assigned to the corresponding categories: "none/minimal" (0–4); "mild" (5–9); "moderate" (10–14); "moderately severe" (15–19); or "severe" (>19). A score of 10 has been recommended as the CO for patients to be referred to expert assessment.

Patient Health Questionnaire for Stress (PHQ-Stress)[35]

The PHQ-Stress is a 10-item subscale of the Primary Care Evaluation of Mental Disorders (PRIME-MD) Patient Health Questionnaire that addresses burden due to psychosocial stress. Patients rate the extent to which they have been bothered by each stressor on a 3-point ordinal rating scale (0: not at all – 2: a lot). Sum scores range from 0 to 20, reflecting the degree of psychosocial stress burden: "none/minimal" (0–4); "mild" (5– 9); "medium" (10–14); or "severe" (>14).

Statistical analysis

Descriptive statistics were calculated to characterize the dataset. A multivariate analysis of variance (MANOVA) was performed, as well as multiple comparisons analysis applying the Bonferroni or Tamhane correction, depending on whether the assumption of the homogeneity of variances was verified. Multiple regressions were performed to determine the cross-sectional associations between each of the independent (predictor) variables (gender, age group, BMI, employment status, typical pain intensity, and respiratory distress) and the dependent (predicted) variable (psychometric scores), expressed by the corresponding regression coefficients. Multiple regression analysis using a stepwise selection was then performed to assess which variables could accurately predict the score of each domain.

All statistical analyses were conducted using IBM SPSS Statistics for Windows, v. 26.0 (IBM Corp., Armonk, USA). Statistical significance was set at $p \leq 0.05$.

Results

Most patients were female (n = 290; 69.9%), with a female to male ratio of 2.32:1, aged 20–59 years (n = 296; 71.3%), workers (n = 246; 59.3%), and had a normal weight (n = 255; 61.4%). A total of 401 patients (96.93%) reported the typical pain intensity associated with their chief pain complaint. On a 11-point numeric rating scale from 0 to 10, the overall mean was 4.82; 161 patients (40.15%) reported typical pain intensity in the range of 4–6 (Table 1).

Table 2 elucidates the descriptive statistics of the study sample grouped by respiratory distress. Most patients did

Table 1. Characteristics of the study sample (N = 415)

Characteristics		n (%)
Gender	F	290 (69.9)
	M	125 (30.1)
Age group [years]	10–19	19 (4.6)
	20–29	68 (16.4)
	30–39	72 (17.3)
	40–49	83 (20.0)
	50–59	73 (17.6)
	60–69	60 (14.5)
	≥70	40 (9.6)
Employment status	training	51 (12.3)
	working	246 (59.3)
	retired	74 (17.8)
	disabled	32 (7.7)
	unemployed	12 (2.9)
BMI	underweight	25 (6.0)
	normal weight	255 (61.4)
	overweight	97 (23.4)
	obesity	38 (9.2)
Typical pain intensity	no answer	14 (3.37)
	0	28 (6.75) **6.98**
	1–3	92 (22.17) **22.94**
	4–6	161 (38.80) **40.15**
	7–10	120 (28.92) **29.93**

BMI – body mass index; F – female; M – male. For typical pain intensity, the percentages are indicated for the total study sample and for the proportion of patients who reported it (in bold).

Table 2. Characteristics of the study sample ($N = 415$) grouped by respiratory distress

| Respiratory distress | Sample | Characteristics | | |

Table 3. Scores of the psychometric measures (a score ≥ cut-off score (CO) indicates clinical relevance)

Domain	*n*	max	*M* ±*SD*	≥CO			
				CO	*n*	% (domain)	% (study sample)
IPQ	331	77/80	42.85 ±11.83	NA	NA	NA	NA
DCQ	45	14/21	5.56 ±4.38	9	14	31.1	3.4
GCPS-H	401	6/6	1.65 ±2.05	3	116	28.9	28.0
GCPS-B	316	6/6	1.04 ±1.72	3	57	18.0	13.7
GAD-7	111	20/21	10.77 ±3.70	10	63	56.8	15.2
IEQ	148	47/48	13.41 ±12.07	18	49	33.1	11.8
PCS	406	49/52	15.94 ±12.91	30	74	18.2	17.3
PHQ-4	415	12/12	2.95 ±3.07	6	82	19.8	19.8
PHQ-9	118	27/27	12.75 ±5.17	10	88	74.6	21.2
PHQ-Stress	111	19/20	6.24 ±3.89	10	18	16.2	4.3

IPQ – Illness Perception Questionnaire; DCQ – Dysmorphic Concern Questionnaire; GCPS-H – Graded Chronic Pain Scale – Head; GCPS-B – Graded Chronic Pain Scale – Body (GCPS-B); GAD-7 – General Anxiety Disorder-7; IEQ – Injustice Experience Questionnaire; PCS – Pain Catastrophizing Scale; PHQ-4 – Patient Health Questionnaire-4; PHQ-9 – Patient Health Questionnaire-9; PHQ-Stress – Patient Health Questionnaire for Stress; *M* – mean; *SD* – standard deviation; NA – not available.

Table 4. Scores of the psychometric measures with regard to the presence (or absence) of respiratory distress (snoring/apnea during sleep)

Domain	Respiratory distress	*n*	*M* ±*SD*	≥CO	
				n	% (domain)
IPQ	yes	79	44.59 ±12.93	NA	NA
	no	252	42.31 ±11.43	NA	NA
DCQ	yes	10	4.50 ±4.22	3	30.00
	no	35	5.86 ±4.43	11	31.43
GCPS-H*	yes	84	2.17 ±2.15	34	40.48
	no	317	1.51 ±1.99	82	25.87
GCPS-B*	yes	70	1.41 ±1.84	19	27.14
	no	246	0.93 ±1.67	38	15.45
GAD-7*	yes	37	12.03 ±3.60	27	72.97
	no	74	10.15 ±3.61	36	48.65
IEQ*	yes	32	19.63 ±15.92	19	59.38
	no	116	11.69 ±10.21	30	25.86
PCS	yes	87	17.49 ±14.02	21	24.14
	no	319	15.52 ±12.78	53	16.61
PHQ-4*	yes	91	3.98 ±3.60	30	32.97
	no	324	2.66 ±2.85	52	16.05
PHQ-9	yes	41	13.73 ±5.36	33	80.49
	no	77	12.22 ±5.02	55	71.43
PHQ-Stress	yes	38	7.08 ±4.50	8	21.05
	no	73	5.81 ±3.49	10	13.70

CO – cut-off score; * indicates the questionnaires where the presence (or absence) of respiratory distress significantly influenced its score (*t* test).

nificant influence on several measures: disabled patients had significantly higher scores as compared to workers on all questionnaires except DCQ and PHQ-9, and on IPQ, GCPS-H, GCPS-B, PCS, and PHQ-4 as compared to trainees. On the other hand, retired patients had significantly lower scores on IPQ, GCPS-H, GCPS-B, PCS, PHQ-4, and PHQ-Stress as compared to disabled persons, and also lower on PHQ-Stress than workers and higher on GCPS-B than trainees. There were no significant effects of BMI on the psychometric variables. Regarding the pain variable, significant effects of typical pain intensity were registered on all questionnaires except DCQ, IEQ, PHQ-9, and PHQ-Stress. The patients who reported a range of 7–10 had significantly higher scores as compared to patients with 0 and 1–3 pain intensity on half of the questionnaires (GCPS-H, GCPS-B, GAD-7, PCS, and PHQ-4, and IPQ, GPCS-H, GPSC-B, PCS, and PHQ-4, respectively). Patients with a reported pain intensity of 4–6 also had lower scores on IPQ and GCPS-H than patients with a pain intensity of 7–10, but significantly higher scores as compared to patients with 0 and 1–3 pain intensity on GCPS-B and PCS, and IPQ, GCPS-H and PCS, respectively. As for respiratory distress, it had a significant influence on GCPS-H, GCPS-B, GAD-7, IEQ and, PHQ-4.

Relationships between (1) gender, age, the employment status, BMI, typical pain intensity, and respiratory distress and (2) the psychometric-domain scores were assessed by multiple regressions (Table 6). The regression coefficients revealed that gender was positively associated with the scores of all questionnaires except IEQ and PHQ-9. This association was statistically significant for GCPS-H and GAD-7, meaning that being female resulted in higher GCPS-H and GAD-7 scores. Age was negatively associated with the score of all questionnaires except IEQ. This association was statistically significant for PHQ-9, meaning older persons had lower scores. The analysis for the employment status should be performed considering the reference category (retired). The positive regression coefficients of the disabled status mean that the psychometric scores were higher for this group than for the retired group, being most statistically significant of these coefficients. The training category exhibited mainly negative

Table 5. Analysis of variance (ANOVA) of the mean psychometric domain scores grouped by gender, age group, employment status, body mass index (BMI), typical pain intensity, and respiratory distress

Psychometric measure	Sample	Characteristics																								
		gender		age group [years]							employment status					BMI				typical pain intensity				respiratory distress	
		F	M	10–19 (A)	20–29 (B)	30–39 (C)	40–49 (D)	50–59 (E)	60–69 (F)	≥70 (G)	training (A)	working (B)	retired (C)	disabled (D)	unemployed (E)	underweight (A)	normal weight (B)	overweight (C)	obesity (D)	0 (A)	1–3 (B)	4–6 (C)	7–10 (D)	yes	no
IPQ	*M*	43.15	42.14	45.23	40.95	44.13	42.51	44.92	40.36	43.49	41.45	41.69	42.80	50.46	50.10	43.27	41.97	44.43	44.15	40.06	37.84	42.36	47.17	44.59	42.31
	ANOVA	NS		NS							(A) vs. (D) ^{*B} (B) vs. (D) ^{**B} (C) vs. (D) ^{*B} F (4,326) = 4.65; p = 0.001					NS				(B) vs. (C) ^{*T} (B) vs. (D) ^{***T} (C) vs. (D) ^{**T} F (3,66) = 7.34; p = 0.000				NS	
DCQ	*M*	5.41	6.25	–	5.17	7.67	6.58	3.29	2.60	5.83	5.67	5.68	4.38	7.67	3.33	7.57	5.12	4.00	12.00	3.00	6.50	4.63	6.39	4.50	5.86
	ANOVA	NS		NS							NS					NS				NS				NS	
GCPS-H	*M*	1.84	1.18	1.72	1.08	2.06	1.78	1.75	1.45	1.65	1.24	1.49	1.73	3.19	1.73	2.32	1.51	1.87	1.50	0.71	0.65	1.61	2.88	2.17	1.51
	ANOVA	**		NS							(A) vs. (D) ^{***B} (B) vs. (D) ^{***B} (C) vs. (D) ^{**B} F (4,396) = 5.63; p = 0.000					NS				(A) vs. (D) ^{***T} (B) vs. (C) ^{***T} (B) vs. (D) ^{***T} (C) vs. (D) ^{***T} F (3,116) = 24.05; p = 0.000				NS	
GCPS-B	*M*	1.10	0.90	0.71	0.70	0.92	0.94	1.53	1.11	1.23	0.49	0.79	1.38	2.83	1.20	1.57	0.84	1.35	1.21	0.13	0.53	1.03	1.56	1.41	0.93
	ANOVA	NS		NS							(A) vs. (C) ^{*T} (A) vs. (D) ^{***T} (B) vs. (D) ^{***T} (C) vs. (D) ^{*T} F (4,46) = 8.11; p = 0.000					NS				(A) vs. (C) ^{***T} (A) vs. (D) ^{***T} (B) vs. (D) ^{***T} F (3,134) = 19.79; p = 0.000				**	
GAD-7	*M*	11.23	9.70	13.25	9.41	10.96	11.14	10.91	10.22	10.75	10.58	10.37	10.78	12.90	8.00	10.29	10.71	10.83	11.71	10.67	8.44	10.49	12.24	12.03	10.15
	ANOVA	*		NS							(B) vs. (D) ^{*B} (D) vs. (E) ^{*B} F (4,106) = 2.98; p = 0.020					NS				(A) vs. (D) ^{**B} F (3,105) = 4.61; p = 0.005				**	
IEQ	*M*	13.39	13.45	16.00	9.04	11.94	14.50	18.79	13.07	11.06	12.00	11.24	13.38	22.10	16.50	13.60	13.14	12.81	15.94	16.00	9.61	13.10	15.14	19.63	11.69
	ANOVA	NS		NS							(B) vs. (D) **B F (4,143) = 3.81; p = 0.006					NS				NS				*	
PCS	*M*	16.47	14.68	17.26	14.65	17.60	17.44	16.97	13.55	13.13	15.54	15.14	14.12	24.97	20.67	18.60	15.29	18.28	17.61	6.89	10.50	16.98	21.26	17.49	15.52
	ANOVA	NS		NS							(A) vs. (D) ^{**B} (B) vs. (D) ^{***B} (C) vs. (D) ^{***B} F (4,401) = 5.12; p = 0.000					NS				(A) vs. (C) ^{***T} (A) vs. (D) ^{***T} (B) vs.(C) ^{***T} (B) vs. (D) ^{***T} F (3,121) = 23.38; p = 0.000				NS	
PHQ-4	*M*	3.07	2.66	3.11	2.82	3.25	3.36	3.42	2.08	2.12	2.94	2.80	2.18	5.56	3.75	3.52	2.85	3.33	2.26	2.14	2.17	3.01	3.84	3.98	2.66
	ANOVA	NS		NS							(A) vs. (D) ^{**T} (B) vs.(D) ^{**T} (C) vs. (D) ^{***T} F (4,57) = 5.47; p = 0.001					NS				(A) vs. (D) ^{*T} (B) vs. (D) ^{***T} F (3,112) = 5.83; p = 0.001				**	

Table 5. – continuation

| Psychometric measure | Sample | Characteristics | | |

Table 6. Effects of the independent (predictor) variables (gender, age, BMI, employment status, typical pain intensity, and respiratory distress) on the dependent (predicted) variable, expressed by the corresponding regression coefficients: Inclusion of all predictor variables (full model)

Dependent variable	Intercept	Gender	Age	Employment status				BMI	Typical pain intensity	Respiratory distress	R^2	ANOVA
				training	working	disabled	unemployed					
IPQ $n = 331$	35.06	0.04	−0.03	−2.50	−0.70	6.00*	6.87	0.09	1.34***	1.23	0.144	F (9,310) = 5.78 $p = 0.000$
DCQ $n = 45$	7.10	0.41	−0.09	−1.62	−1.65	2.10	−3.12	0.14	0.14	−1.71	0.157	F (9,35) = 0.73 $p = 0.682$
GCPS-H $n = 401$	0.61	0.41*	−0.007	−0.86	−0.21	1.00*	−0.10	−0.01	0.29***	0.56*	0.222	F (9,391) = 12.37 $p = 0.000$
GCPS-B $n = 316$	0.65	0.13	−0.004	−1.07*	−0.55	1.28**	−0.27	−0.003	0.17***	0.28	0.200	F (9,301) = 8.37 $p = 0.000$
GAD-7 $n = 111$	3.00	1.90*	−0.01	1.17	0.36	2.55	−1.47	0.18	0.29*	1.79**	0.245	F (9,99) = 3.57 $p = 0.001$
IEQ $n = 148$	8.64	−1.31	0.10	2.72	1.86	9.10*	6.18	−0.25	0.53	7.73**	0.171	F (9,137) = 3.13 $p = 0.002$
PCS $n = 406$	9.19	0.51	−0.08	−2.27	0.06	7.61**	5.21	0.04	1.78***	1.20	0.187	F (9,391) = 9.99 $p = 0.000$
PHQ-4 $n = 415$	2.88	0.22	−0.02	−0.004	0.40	2.56***	1.22	−0.05	0.24***	1.30***	0.149	F (9,391) = 7.59 $p = 0.000$
PHQ-9 $n = 118$	15.98	−0.66	−0.13***	−5.07*	−1.26	1.37	−1.14	0.05	0.49**	1.54	0.239	F (9,107) = 3.73 $p = 0.000$
PHQ-Stress $n = 111$	7.35	0.86	−0.06	−0.06	1.28	5.54***	0.38	−0.06	0.09	0.86	0.278	F (9,99) = 4.23 $p = 0.000$

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. Positive (negative) coefficients mean that higher values of the independent variables are associated with higher (lower) values of the dependent variable. Patients' gender was coded with 0 (male) and 1 (female). The employment status was coded categorically, using "retired" as the reference category and including all other categories (training, working, disabled, and unemployed) in the equation. Respiratory distress was coded 0 (absence) and 1 (presence). The sample size used in each regression is indicated by *n*.

Table 7. Effects of the independent (predictor) variables (gender, age, BMI, employment status, typical pain intensity, and respiratory distress) on the dependent (predicted) variable, expressed by the corresponding regression coefficients: Selection of the best combination of predictor variables to explain the predicted variable (stepwise model)

Dependent variable	Intercept	Gender	Age	Employment status				BMI	Typical pain intensity	Respiratory distress	R^2	ANOVA
				training	working	disabled	unemployed					
IPQ $n = 331$	35.31	–	–	–	–	7.13***	7.67*	–	1.33***	–	0.138	F (3,316) = 16.84 $p = 0.000$
GCPS-H $n = 401$	−0.26	0.43*	–	–	–	1.24***	–	–	0.29***	0.53*	0.213	F (4,396) = 26.84 $p = 0.000$
GCPS-B $n = 316$	0.01	–	–	–	–	1.84***	–	–	0.17***	–	0.177	F (2,308) = 33.07 $p = 0.000$
GAD-7 $n = 111$	8.00	–	–	–	–	1.89*	–	–	0.34**	1.90**	0.176	F (3,105) = 7.46 $p = 0.000$
IEQ $n = 148$	10.84	–	–	–	–	8.25**	–	–	–	7.03**	0.139	F (2,144) = 11.67 $p = 0.000$
PCS $n = 406$	6.85	–	–	–	–	7.81***	–	–	1.78***	–	0.171	F (2,398) = 41.15 $p = 0.000$
PHQ-4 $n = 415$	2.27	–	−0.02*	–	–	2.28***	–	–	0.24***	1.20***	0.137	F (4,396) = 15.69 $p = 0.000$
PHQ-9 $n = 118$	15.45	–	−0.11***	−4.09**	–	2.63*	–	–	0.45**	–	0.205	F (4,112) = 7.21 $p = 0.000$
PHQ-Stress $n = 111$	8.27	–	−0.06**	–	–	4.89***	–	–	–	–	0.235	F (2,106) = 16.29 $p = 0.000$

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. Statistical criteria for the inclusion of the independent variables in the regression equation: probability to enter ≤ 0.050; probability to remove ≥ 0.100. Patients' gender was coded with 0 (male) and 1 (female). The employment status was coded categorically, using "retired" as the reference category and including all other categories (training, working, disabled, and unemployed) in the equation. Respiratory distress was coded 0 (absence) and 1 (presence). The sample size used in each regression is indicated by *n*.

Characterization of the study sample

We analyzed the impact of sleep-disordered breathing (SDB) in a large sample of 415 patients seeking care at an OFP unit, covering a broad age range. An initial comprehensive evaluation of all patients who completed WISE (Table 1). The gender proportion and age distribution of our study sample are similar to the ones reported in previous studies on patients seeking care for OFP.[36] The majority of the patients were workers (n = 246; 59.3%) and a small percentage of them were disabled, either because of an illness or an accident (n = 32; 7.7%). In accordance with the trend observed in Switzerland during the last few years,[37] more than half of the patients presented a normal weight (n = 255; 61.4%) and only 9.2% of the participants (n = 38) were obese. These results represent the wave of change observed in this country, which had the lowest prevalence of obesity in the years 2011–2016.[37] Regarding the pain variable, on a 11-point numeric pain scale, the reported mean for typical pain intensity was moderate (4.82). Orofacial is one of the most common reasons for patients to seek treatment.[38] Furthermore, patients with OFP or TMD frequently suffer from other comorbid pain symptoms.[39] In line with this, most patients classified their typical pain intensity in a range between 4 and 6 (n = 161; 40.15%).

As WISE is a modular and flexible tool, several items can be added to it depending on the needs of a given clinic or research focus. Regarding this study, an item corresponding to snoring or apnea during sleep was already included, and allowed us to group the sample according to the presence or absence of these symptoms (Table 2). Even though both sleep apnea and snoring are generally more common in males,[8,22] our results showed that more than half of the patients suffering from these conditions were females (n = 57; 62.6%). This may be influenced by the fact that this clinical population refers to people who reported to a pain unit, therefore being mostly composed of women. However, when analyzing the percentage of SRBD among each gender, this was higher for males (n = 34; 27.2%) as compared to females (n = 57; 19.7%). As for the age range, most people reporting sleep-related respiratory distress were 40–59 years old. Considering that OSA is more dominant in middle-aged individuals[22] and that the overall prevalence of snoring increases with age,[8] our results highlight this age range as the most troubled. Additionally, the prevalence of snoring and OSA in adults is reported to reach a peak at the age of 40–60 years.[3] The employment status was included as a relevant variable to characterize our sample, given the impact of SDB on work capability and its socioeconomic burden.[31,40] Most patients reporting snoring or sleep apnea were workers (n = 56; 61.5%), and retired people reported the second highest prevalence (n = 15; 16.5%), reflecting the underlying factor of age. The body mass index was also taken into consideration, as obesity is the most important reversible risk factor for OSA,[41] and snoring has previously been associated with BMI.[8] Approximately 60% of the group with respiratory distress reported a normal weight, and only 10% reported obesity. Moreover, Kerner and Roose reported in 2016 that the impact of BMI on OSA severity decreases with age.[42] The fact that the percentage of people with SRBD was the highest among underweight patients (relative percentage: 28%) further reinforces a weak relationship between BMI and sleep-related respiratory distress in our study sample.

We further analyzed the group which suffered more from sleep-related respiratory distress according to gender and age range. This group encompassed women aged 50–59 years (n = 19; 4.6%), and for the same age range, the percentage for men was 1.7% (n = 7) (Fig. 1). This age range is of particular interest for women, as menopause typically occurs between the age of 40 and 60 years, and the literature shows important differences in the prevalence and symptoms of SDB between pre- and post-menopausal women.[43,44]

Psychometric assessment

In this study, we used a variety of features as measures of psychosocial stress, such as depression, anxiety, mental health, distress, and pain features, which are commonly associated with poor well-being.[45] Among the numerous relatively brief self-report questionnaires that have been developed and validated to assess psychosocial stress, the ones presented herein were used in WISE, and serve as proxies for well-being.

The number of patients who completed at least one of the case-finding questionnaires ranged from 45 (DCQ) to 415 (PHQ-4) (Table 3). As PHQ-4 is part of the WISE checklist, all patients completed it, and approx. 20% of the study sample scored above the cut-off, indicating the need for further expert evaluation. The sample prevalence of clinically relevant psychometric scores was in descending order: 28.0% (GCPS-H); 21.2% (PHQ-9); 19.8% (PHQ-4); 17.8% (PCS); 15.2% (GAD-7); 13.7% (GCPS-B); 11.8% (IEQ); 4.3% (PHQ-Stress); and 3.4% (DCQ). No cut-off value has been reported for IPQ. About 36% of the study sample had clinically relevant scores for GAD-7 and PHQ-9, which means that approx. 1 in 3 patients had potential impairment associated with general anxiety and depression assessed by those 2 questionnaires, warranting further clinical assessment. Interestingly, only a relatively small percentage of the study sample reported a medium or severe burden by psychosocial stress, assessed by PHQ-Stress (4.3%). About 1 in 4 patients experienced moderate to severe limitation due to craniofacial pain, and about 14% due to pain in other body parts. Considering our specific clinical population, nearly 18% of patients reported pain catastrophizing scores above the cut-off value, which relates to the percentage reported in a similar previous study.[36] To bet-

ter understand these results, a further assessment of the psychometric scores was performed according to the presence or absence of sleep-related respiratory distress.

Table 4 indicates that for all domains, the percentage of patients reaching a clinically relevant score was higher for the group with respiratory distress, except for DCQ, where the percentages for the 2 groups were similar. Furthermore, the presence of respiratory distress was found to significantly influence the scores of GCPS-H, GCPS-B, GAD-7, IEQ, and PHQ-4. Our results are supported by current literature, which has shown that QoL is impaired in OSA patients as compared to non-OSA patients,[46] and that the association between OSA and depression and anxiety is clinically relevant.[15] Indeed, the QoL of OSA patients and their bed partners is shown to improve when sleep apnea is successfully treated.[46] As for snoring, even though its clinical relevance is yet to be fully clarified,[7] the risk of daytime anxiety and the risk of depression for both snorers and their sleeping partners are reported to be increased.[8] It should be noted that these relationships have been found both in adult and pediatric populations.[22] The influence of respiratory distress on the measures of persistent pain was not surprising, as SDB can be related to various types of pain, such as headache.[47] In fact, GCPS-related disability scoring has been suggested as a simple screening instrument to identify TMD patients with different degrees of health burdens (e.g., sleep conditions), correlated with general well-being.[48] The IEQ is mainly related to the presence of an injury. However, considering that it contributes to prolonged emotional stress, we recognize it can be related to poor sleep quality, which may partly explain our results. Various lines of evidence also point to an increased prevalence of psychosis and dementia in patients with sleep apnea.[49]

Psychometric assessment according to the sample characteristics

The performed analysis of variance allowed us to further understand the effect of respiratory distress, gender, age, the employment status, and BMI on the psychometric scores for the entire study population (Table 5).

Great efforts have been made to clarify sex differences in emotional processing, being now widely accepted that women experience emotions differently than men. Many studies focusing on the association between gender and depression in OSA patients, for instance, established that depression was more prevalent in females than in males, with the same tendency observed for anxiety symptoms.[15] In our study, women suffered more general anxiety than men, which supports the abovementioned notion. No significant effects of age on the psychometric measures were observed, except that older patients (>70 years) had lower PHQ-Stress scores as compared to those aged 20–59 years. This is in line with a recent paper which reported low PHQ scores in young adulthood, an increase during middle age, followed by a new decrease in the scores for older age.[20] Regarding the effect of the employment status, several measures were higher for disabled patients as compared to workers (all questionnaires except DCQ and PHQ-9) and trainees (IPQ, GCPS-H, GCPS-B, PCS, and PHQ-4), while workers reported higher PHQ-Stress scores in comparison with retired people. Retired people also had significantly lower scores on IPQ, GCPS-H, GCPS-B, PCS, PHQ-4, and PHQ-Stress as compared to disabled persons, and higher on GCPS-B than trainees. Even though the relationship between these employment conditions and psychosocial stress is still to be refined, these results highlight the need for clarifying the impact of employment-related factors. The literature shows that low income, unemployment and professional requirements are often associated with sleep problems, ultimately affecting the individual's QoL.[19] A better recognized relation is described for the PHQ scores, with OSA workers reporting higher job-related stress.[50] Nevertheless, considering that disabled patients suffered from an illness or accident in the past, which prevents them from working and affects their daily activities, it is not surprising that this status was associated with a higher psychosocial burden. What is surprising is that BMI had no significant effect on the psychometric scores, going against a recent study which found a strong positive correlation between snoring and BMI in both sexes.[8] However, we should have in mind the characteristics of our study sample, which was relatively healthy as discussed above. The significant effect of typical pain intensity on the scores of most questionnaires met our expectations, and is supported by the relationship described in the literature between pain intensity, pain-related disability and QoL.[38]

As for respiratory distress, the analysis of variance supported the previous findings of the influence of respiratory distress on the GCPS-H, GCPS-B, GAD-7, IEQ, and PHQ-4 scores. The statistical significance of these relationships is further explored in the next section.

Associations between respiratory distress and the well-being scores

Relationships between the independent variables, including not only respiratory distress, but also gender, age, the employment status, BMI, and typical pain intensity, and the dependent variable (the psychometric scores), were examined by multiple regression analysis. The directionality of the effects of each variable is presented in Table 6, whereas the best combination of predictor variables for each questionnaire is displayed in Table 7.

The key finding of our analysis was that respiratory distress was positively associated with the scores of all domains except DCQ, being a statistically significant

predictor for GCPS-H, GAD-7, IEQ, and PHQ-4. These observations are based on the respective *p*-values. The findings of these regression analyses further support the relation described in previous epidemiologic studies, which have consistently demonstrated that SDB is associated with depression and anxiety symptoms.[15,42] On the other hand, they confirm the existence of an association between pain and sleep disturbance, which has been a focus of research since the 1990s, when Affleck and colleagues reported a bidirectional link between pain perception and sleep quality.[51] The fact that SDB may be a frequent cause of headaches, which was a checklist item included in WISE, may justify why respiratory distress was found to be predictive of pain-related disability due to pain in the head.[44] Moreover, current literature provides evidence that SDB tends to exacerbate persistent pain, particularly craniofacial pain, which affects sleep quality.[47,52] Likewise, hypoxemic-related mechanisms seem to be involved in such a relationship, also impacting critical cardiometabolic aspects.[53] Whether vitamin D, electrolytic balance and uric acid concentration could be related to that dynamics has also been a matter of discussion with potentially important clinical implications.[54] Gender was found to have a statistically significant relation only with GCPS-H. Given the existence of gender differences in pain sensitivity and processing, with women seeking treatment more often than men, its impact is well-supported.[54,55] In fact, the female gender shows a higher prevalence for almost all pain problems affecting the head, neck and OFP regions.[56] The positive coefficients of the disabled status for all domains highlight this condition as determinant for worsened psychosocial well-being as compared to retired people. Considering that the unemployed and training status also showed significant coefficients, our findings emphasize the need to screen for employment status information to optimize clinical management. Typical pain intensity was a significant predictor of the scores of all questionnaires except DCQ, IEQ and PHQ-Stress. Temporomandibular disorders and OFP share certain features and are associated with chronic pain conditions, such as tension headaches and fibromyalgia, being characterized by biopsychosocial stress and complaints, including fatigue, anxiety and depression.[57] As for age, it was found to be a statistically significant predictor for PHQ-4, PHQ-9 and PHQ-Stress. The fact that some of these predictors were absent in certain models should not be a reason for discrediting the previous results. This may be explained by a small sample size or the need for a different model to observe those relations. Nonetheless, the models presented herein underscore the influence of the selected variables on the psychometric measures. Within this context, the negative psychosocial impact of abnormal respiratory patterns on the patient's well-being should be considered in light of other confounding factors, such as age, gender and pain features.

Limitations

The present study has several limitations that must be considered when interpreting our results. As is common in cross-sectional studies, causality cannot be inferred from the observed relationship between psychosocial stress and sleep-related respiratory distress. Another limitation is our reliance on self-reported snoring and sleep apnea, which may have introduced reporting bias. Additionally, due to the structure of WISE, we did not distinguish between patients with simple snoring and those with sleep apnea, nor did we assess disease severity.

Although OSA is common and should be suspected in all sleepy snorers, it is well established that a proportion of individuals with OSA do not snore, and not all snorers have coexisting OSA. Furthermore, caution is warranted when generalizing our findings to a broader population, as our sample consisted of a clinical population presenting to an OFP/TMD unit. Moreover, children and adolescents were assessed using instruments primarily developed for adult populations. Therefore, future studies should aim to include broader and more representative samples, as well as age-appropriate, validated assessment tools for younger populations.

These limitations should be carefully considered in future research investigating SDB and psychosocial stress. Despite these constraints, the present findings have important practical implications, as they underscore the value of systematically assessing sleep-related variables in clinical evaluations.

Conclusions

In this study cross-sectional study, about 22% of the evaluated patients reported some form of SRRD. Moreover, SRBD were associated with higher psychometric scores, suggesting a close relationship between sleep related respiratory difficulties and psychosocial impairment.

Ethics approval and consent to participate

According to the Swiss law, the analysis of strictly anonymized data does not require the approval by an ethics committee. Only data from patients who had given consent to the use of their anonymized data for research was used.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Artificial intelligence (AI) was used as an adjunctive tool for English language editing and for the preparation of the graphical abstract. The authors reviewed and take full responsibility for the final content.

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References

1. Santos I, Rocha I, Gozal D, Meira e Cruz M. Obstructive sleep apnea, shift work and cardiometabolic risk. *Sleep Med*. 2020;74:132–140. doi:10.1016/j.sleep.2020.05.013
2. Biggs SN, Walter LM, Jackman AR, et al. Longitudinal impact of resolution of snoring in young children on psychosocial functioning. *J Pediatr*. 2015;167(6):1272–1279. doi:10.1016/j.jpeds.2015.09.016
3. Turnbull C, Zaki I, Hardinge M. Obstructive sleep apnoea. *Medicine*. 2020;48(6):404–411. doi:10.1016/j.mpmed.2020.03.008
4. Ramar K, Dort LC, Katz SG, et al. Clinical practice guideline for the treatment of obstructive sleep apnea and snoring with oral appliance therapy: An update for 2015. *J Clin Sleep Med*. 2015;11(7):773–827. doi:10.5664/jcsm.4858
5. Zhang Y, Ren R, Lei F, et al. Worldwide and regional prevalence rates of co-occurrence of insomnia and insomnia symptoms with obstructive sleep apnea: A systematic review and meta-analysis. *Sleep Med Rev*. 2019;45:1–17. doi:10.1016/j.smrv.2019.01.004
6. Meira e Cruz M, Soca R, Kryger M. How much is too much after all? Primary snoring as a remaining unsolved issue. *J Clin Sleep Med*. 2020;16(6):991. doi:10.5664/jcsm.8442
7. Macarthur KE, Bradley TD, Ryan CM, Alshaer H. Dissociation between objectively quantified snoring and sleep quality. *Am J Otolaryngol*. 2020;41(1):102283. doi:10.1016/j.amjoto.2019.102283
8. Campos AI, García-Marín LM, Byrne EM, Martin NG, Cuéllar-Partida G, Rentería ME. Insights into the aetiology of snoring from observational and genetic investigations in the UK Biobank. *Nat Commun*. 2020;11(1):817. doi:10.1038/s41467-020-14625-1
9. Vanek J, Prasko J, Genzor S, et al. Obstructive sleep apnea, depression and cognitive impairment. *Sleep Med*. 2020;72:50–58. doi:10.1016/j.sleep.2020.03.017
10. Kales A, Caldwell AB, Cadieux RJ, Vela-Bueno A, Ruch LG, Mayes SD. Severe obstructive sleep apnea – II: Associated psychopathology and psychosocial consequences. *J Chronic Dis*. 1985;38(5):427–434. doi:10.1016/0021-9681(85)90138-9
11. Schwarz J, Gerhardsson A, Van Leeuwen W, et al. Does sleep deprivation increase the vulnerability to acute psychosocial stress in young and older adults? *Psychoneuroendocrinology*. 2018;96:155–165. doi:10.1016/j.psyneuen.2018.06.003
12. Hackett RA, Steptoe A. Psychosocial factors in diabetes and cardiovascular risk. *Curr Cardiol Rep*. 2016;18(10):95. doi:10.1007/s11886-016-0771-4
13. Jang HH, Kim ME, Kim HK. Pain catastrophizing mediates the effects of psychological distress on pain interference in patients with orofacial pain: A cross-sectional study. *J Oral Facial Pain Headache*. 2018;32(4):409–417. doi:10.11607/ofph.2067
14. Gupta MA, Simpson FC. Obstructive sleep apnea and psychiatric disorders: A systematic review. *J Clin Sleep Med*. 2015;11(2):165–175. doi:10.5664/jcsm.4466
15. Garbarino S, Bardwell WA, Guglielmi O, Chiorri C, Bonnani E, Magnavita N. Association of anxiety and depression in obstructive sleep apnea patients: A systematic review and meta-analysis. *Behav Sleep Med*. 2020;18(1):35–57. doi:10.1080/15402002.2018.1545649
16. Aronen ET, Liukkonen K, Simola P, et al. Mood is associated with snoring in preschool-aged children. *J Dev Behav Pediatr*. 2009;30(2):107–114. doi:10.1097/DBP.0b013e31819d70a2
17. Bahammam AS, Kendzerska T, Gupta R, et al. Comorbid depression in obstructive sleep apnea: An under-recognized association. *Sleep Breath*. 2016;20(2):447–456. doi:10.1007/s11325-015-1223-x
18. Fillingim RB, Slade GD, Greenspan JD, et al. Long-term changes in biopsychosocial characteristics related to temporomandibular disorder: Findings from the OPPERA study. *Pain*. 2018;159(11):2403–2413. doi:10.1097/j.pain.0000000000001348
19. Garbarino S, Lanteri P, Durando P, Magnavita N, Sannita WG. Co-morbidity, mortality, quality of life and the healthcare/welfare/social costs of disordered sleep: A rapid review. *Int J Environ Res Public Health*. 2016;13(8):831. doi:10.3390/ijerph13080831
20. Tomitaka S, Kawasaki Y, Ide K, Akutagawa M, Ono Y, Furukawa TA. Stability of the distribution of Patient Health Questionnaire-9 scores against age in the general population: Data from the National Health and Nutrition Examination Survey. *Front Psychiatry*. 2018;9:390. doi:10.3389/fpsyt.2018.00390
21. Goldfarb EV, Seo D, Sinha R. Sex differences in neural stress responses and correlation with subjective stress and stress regulation. *Neurobiol Stress*. 2019;11:100177. doi:10.1016/j.ynstr.2019.100177
22. Horne RS, Ong C, Weichard A, Nixon GM, Davey MJ. Are there gender differences in the severity and consequences of sleep disordered in children? *Sleep Med*. 2020;67:147–155. doi:10.1016/j.sleep.2019.11.1249
23. Ettlin DA, Sommer I, Brönnimann B, et al. Design, construction, and technical implementation of a web-based interdisciplinary symptom evaluation (WISE) – a heuristic proposal for orofacial pain and temporomandibular disorders. *J Headache Pain*. 2016;17(1):77. doi:10.1186/s10194-016-0670-5
24. Boggero IA, Rojas-Ramirez MV, De Leeuw R, Carlson CR. Satisfaction with life in orofacial pain disorders: Associations and theoretical implications. *J Oral Facial Pain Headache*. 2016;30:99–106. doi:10.11607/ofph.1526
25. Wong MC, McMillan AS, Zheng J, Lam CL. The consequences of orofacial pain symptoms: A population-based study in Hong Kong. *Community Dent Oral Epidemiol*. 2008;36(5):417–424. doi:10.1111/j.1600-0528.2007.00422.x
26. Galli U, Ettlin DA, Palla S, Ehlert U, Gaab J. Do illness perceptions predict pain-related disability and mood in chronic orofacial pain patients? A 6-month follow-up study. *Eur J Pain*. 2010;14(5):550–558. doi:10.1016/j.ejpain.2009.08.011
27. Broadbent E, Petrie KJ, Main J, Weinman J. The Brief Illness Perception Questionnaire. *J Psychosom Res*. 2006;60(6):631–637. doi:10.1016/j.jpsychores.2005.10.020
28. Mancuso SG, Knoesen NP, Castle DJ. The Dysmorphic Concern Questionnaire: A screening measure for body dysmorphic disorder. *Aust N Z J Psychiatry*. 2010;44(6):535–542. doi:10.3109/00048671003596055
29. Von Korff M, Ormel J, Keefe FJ, Dworkin SF. Grading the severity of chronic pain. *Pain*. 1992;50(2):133–149. doi:10.1016/0304-3959(92)90154-4
30. Löwe B, Decker O, Müller S, et al. Validation and standardization of the Generalized Anxiety Disorder Screener (GAD-7) in the general oopulation. *Med Care*. 2008;46(3):266–274. doi:10.1097/MLR.0b013e318160d093
31. Sullivan MJ, Adams H, Horan S, Maher D, Boland D, Gross R. The role of perceived injustice in the experience of chronic pain and disability: Scale development and validation. *J Occup Rehabil*. 2008;18(3):249–261. doi:10.1007/s10926-008-9140-5
32. Sullivan MJ, Bishop SR, Pivik J. The Pain Catastrophizing Scale: Development and validation. *Psychol Assess*. 1995;7(4):524–532. doi:10.1037/1040-3590.7.4.524
33. Löwe B, Wahl I, Rose M, et al. A 4-item measure of depression and anxiety: Validation and standardization of the Patient Health Questionnaire-4 (PHQ-4) in the general population. *J Affect Disord*. 2010;122(1–2):86–95. doi:10.1016/j.jad.2009.06.019

34. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: Validity of a brief depression severity measure. *J Gen Intern Med*. 2001;16(9):606–613. doi:10.1046/j.1525-1497.2001.016009606.x
35. Spitzer RL, Williams JB, Kroenke K, Hornyak R, McMurray J. Validity and utility of the PRIME-MD Patient Health Questionnaire in assessment of 3000 obstetric-gynecologic patients: The PRIME-MD Patient Health Questionnaire Obstetrics-Gynecology Study. *Am J Obstet Gynecol*. 2000;183(3):759–769. doi:10.1067/mob.2000.106580
36. Bhalang K, Steiger B, Lukic N, Zumbrunn Wojczyńska A, Hovijitra RS, Ettlin DA. The pain-to-well-being relationship in patients experiencing chronic orofacial pain. *Front Neurol*. 2020;11:557415. doi:10.3389/fneur.2020.557415
37. Garrido-Miguel M, Cavero-Redondo I, Álvarez-Bueno C, et al. Prevalence and trends of overweight and obesity in European children from 1999 to 2016: A systematic review and meta-analysis. *JAMA Pediatr*. 2019;173(10):e192430. doi:10.1001/jamapediatrics.2019.2430
38. Oghli I, List T, Su N, Häggman-Henrikson B. The impact of oro-facial pain conditions on oral health-related quality of life: A systematic review. *J Oral Rehabil*. 2020;47(8):1052–1064. doi:10.1111/joor.12994
39. Cioffi I, Perrotta S, Ammendola L, et al. Social impairment of individuals suffering from different types of chronic orofacial pain. *Prog Orthod*. 2014;15(1):27. doi:10.1186/s40510-014-0027-z
40. Jennum P, Kjellberg J. Health, social and economical consequences of sleep-disordered breathing: A controlled national study. *Thorax*. 2011;66(7):560–566. doi:10.1136/thx.2010.143958
41. Trakada G, Chrousos G, Pejovic S, Vgontzas A. Sleep apnea and its association with the stress system, inflammation, insulin resistance and visceral obesity. *Sleep Med Clin*. 2007;2(2):251–261. doi:10.1016/j.jsmc.2007.04.003
42. Kerner NA, Roose SP. Obstructive sleep apnea is linked to depression and cognitive impairment: Evidence and potential mechanisms. *Am J Geriatr Psychiatry*. 2016;24(6):496–508. doi:10.1016/j.jagp.2016.01.134
43. Honour JW. Biochemistry of the menopause. *Ann Clin Biochem*. 2018;55(1):18–33. doi:10.1177/0004563217739930
44. Perger E, Mattaliano P, Lombardi C. Menopause and sleep apnea. *Maturitas*. 2019;124:35–38. doi:10.1016/j.maturitas.2019.02.011
45. Hall LH, Johnson J, Watt I, Tsipa A, O'Connor DB. Healthcare staff wellbeing, burnout, and patient safety: A systematic review. *PLoS One*. 2016;11(7):e0159015. doi:10.1371/journal.pone.0159015
46. Conte L, Toraldo DM, Arigliani MM, Greco M, Maffia M, De Benedetto M. Obstructive sleep apnea (OSA), an emerging health problem. *J Interdiscip Res Appl Med*. 2019;3(2):7–16. doi:10.1285/i25327518v3i2p7
47. Marshansky S, Mayer P, Rizzo D, Baltzan M, Denis R, Lavigne GJ. Sleep, chronic pain, and opioid risk for apnea. *Prog Neuropsychopharmacol Biol Psychiatry*. 2018;87 (Pt B):234–244. doi:10.1016/j.pnpbp.2017.07.014
48. Kotiranta U, Forssell H, Kauppila T. Painful temporomandibular disorders (TMD) and comorbidities in primary care: Associations with pain-related disability. *Acta Odontol Scand*. 2019;77(1):22–27. doi:10.1080/00016357.2018.1493219
49. Sharafkhaneh A, Giray N, Richardson P, Young T, Hirshkowitz M. Association of psychiatric disorders and sleep apnea in a large cohort. *Sleep*. 2005;28(11):1405–1411. doi:10.1093/sleep/28.11.1405
50. Guglielmi O, Jurado-Gámez B, Gude F, Buela-Casal G. Job stress, burnout, and job satisfaction in sleep apnea patients. *Sleep Med*. 2014;15(9):1025–1030. doi:10.1016/j.sleep.2014.05.015
51. Affleck G, Urrows S, Tennen H, Higgins P, Abeles M. Sequential daily relations of sleep, pain intensity, and attention to pain among women with fibromyalgia. *Pain*. 1996;68(2–3):363–368. doi:10.1016/s0304-3959(96)03226-5
52. Carra MC, Bruni O, Huynh N. Topical review: Sleep bruxism, headaches, and sleep-disordered breathing in children and adolescents. *J Orofac Pain*. 2012;26(4):267–276. PMID:23110266.
53. Więckiewicz M, Lavigne G, Martynowicz H. Decrypting the putative interrelation between sleep bruxism, masticatory muscle pain and sleep breathing disorders: Nosology and the role of hypoxia. *Dent Med Probl*. 2024;61(2):165–167. doi:10.17219/dmp/175686
54. Kanclerska J, Wieckiewicz M, Nowacki D, et al. Sleep architecture and vitamin D in hypertensives with obstructive sleep apnea: A polysomnographic study. *Dent Med Probl*. 2024;61(1):43–52. doi:10.17219/dmp/172243
55. Shinal RM, Fillingim RB. Overview of orofacial pain: Epidemiology and gender differences in orofacial pain. *Dent Clin North Am*. 2007;51(1):1–18. doi:10.1016/j.cden.2006.09.004
56. Shaefer JR, Khawaja SN, Bavia PF. Sex, gender, and orofacial pain. *Dent Clin North Am*. 2018;62(4):665–682. doi:10.1016/j.cden.2018.06.001
57. Sommer I, Lavigne G, Ettlin DA. Review of self-reported instruments that measure sleep dysfunction in patients suffering from temporomandibular disorders and/or orofacial pain. *Sleep Med*. 2015;16(1):27–38. doi:10.1016/j.sleep.2014.07.023

Original paper

Disproportionality analysis of oral adverse events following COVID-19 vaccination: Investigating demographic disparities and notoriety bias using a hybrid frequentist–Bayesian approach

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Abstract

Background. Ensuring vaccine safety is crucial for maintaining public confidence, particularly in relation to non-life-threatening adverse events (AEs) that may impact quality of life and contribute to vaccine hesitancy. While systemic reactions to coronavirus disease 2019 (COVID-19) vaccines are well documented, oral AEs remain underexplored, despite emerging reports linking vaccination to taste disturbances and other oral manifestations.

Objectives. The aim of the study was to explore oral AEs following COVID-19 vaccination and to assess their potential to constitute signals of disproportionate reporting (SDRs) that warrant further investigation and validation.

Material and methods. A hybrid frequentist–Bayesian signal detection approach was applied to analyze oral AEs following COVID-19 vaccination using data from the US Vaccine Adverse Event Reporting System (VAERS). A two-stage analysis was conducted. The primary analysis identified SDRs, and the secondary analysis assessed reporting biases, including selective reporting bias and notoriety bias.

Results. Oral AEs were reported 2.4 times more frequently following COVID-19 vaccination compared with other vaccines, with taste-related SDRs showing the highest disproportionality. The most prominent SDRs included taste disorder (proportional reporting ratio (*PRR*) = 23.96), ageusia (*PRR* = 17.79), hypogeusia (*PRR* = 6.25), dysgeusia (*PRR* = 3.22), and oral lichen planus (*PRR* = 3.12). Females and middle-aged adults exhibited the highest reporting rates, whereas severe, hospitalized and fatal cases were less frequent. Co-occurrence analysis of gustatory and olfactory AEs suggests notoriety bias, likely influenced by increased public awareness. Regression analyses indicated that female sex and older age were associated with increased odds of oral SDRs, while state-level political inclination had no significant effect.

Conclusions. The findings highlight demographic disparities in the reporting of oral AEs. However, they should not be interpreted as undermining vaccine safety. Future research should focus on clinically significant oral SDRs, such as oral lichen planus, to distinguish true safety signals from potential reporting biases. Improved awareness and communication are essential for contextualizing these findings within dental practice.

Keywords: COVID-19 vaccines, oral manifestations, pharmacovigilance, adverse drug reaction reporting systems, Bayesian analysis

Highlights

- Oral adverse events (AEs) were reported 2.4 times more frequently after COVID-19 vaccination than after other vaccines in the Vaccine Adverse Event Reporting System (VAERS), indicating signals of disproportionate reporting.
- The highest disproportionality was observed for taste disorder (proportional reporting ratio (*PRR*) ≈ 23.96), ageusia (≈ 17.79), hypogeusia (≈ 6.25), and dysgeusia (≈ 3.22), along with other sensory- and dental-related AEs.
- Higher reporting ratios were noted among females than males, while severe, hospitalized and fatal cases were relatively uncommon, suggesting that most oral AEs were mild.
- The frequent co-occurrence of gustatory and olfactory AEs, together with pandemic-related temporal patterns, suggests that increased public awareness may have influenced reporting, highlighting the need to distinguish true safety signals from stimulated reporting.
- Similar disproportionality of oral AEs was observed in "red" and "blue" states, and state-level political inclination was not a significant predictor in regression analyses, indicating that reporting patterns were largely independent of political context.

Introduction

Throughout history, anti-vaccination movements have actively spread misinformation regarding vaccine safety, often amplifying reports of adverse events (AEs) to undermine public confidence in immunization programs.[1,2] These tactics have evolved with advancements in communication technologies, particularly through social media, leading to widespread vaccine hesitancy.[3] To counteract misinformation and ensure public trust, national pharmacovigilance systems play a crucial role in monitoring post-vaccination AEs, identifying safety signals and distinguishing true vaccine-related risks from coincidental events.[4,5] The Vaccine Adverse Event Reporting System (VAERS) in the United States serves as a cornerstone of passive surveillance, allowing healthcare providers and the public to report potential vaccine-related AEs.[6] Among these, oral manifestations such as taste disorders, paresthesia and ulcers have emerged as notable concerns following coronavirus disease 2019 (COVID-19) vaccination.[7–11]

A growing body of evidence suggests that certain AEs may be disproportionately reported due to increased public awareness, regulatory alerts or media attention, a phenomenon known as notoriety bias.[4,12] This bias can lead to an overestimation of AE incidence in passive surveillance databases, as individuals become more likely to report events they perceive as vaccine-related. Taste-related AEs are particularly susceptible to notoriety bias, as loss of taste and smell are widely recognized diagnostic symptoms of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.[13–15] Previous studies have shown that the reporting rate of taste-related AEs in VAERS was from 3 to 17 times higher during the pandemic compared with the pre-pandemic period.[7] This raises concerns about whether taste-related AEs following COVID-19 vaccination reflect a true biological association or are influenced by heightened public vigilance.

While numerous studies have examined AEs following COVID-19 vaccination, most have focused on systemic reactions such as fever, fatigue and anaphylaxis, with relatively little attention given to less common AEs, including oral manifestations.[16,17] Prior analyses of passive surveillance databases have identified common patterns of oral AEs in major regions, such as the United States, the European Union and Australia; however, no study has comprehensively assessed their disproportionality using advanced signal detection methods.[9]

The present study aimed to conduct a disproportionality analysis of oral AEs following COVID-19 vaccination using a hybrid frequentist–Bayesian signal detection approach, thereby contributing to the long-term pharmacovigilance of novel vaccine technologies that remain in global use. By identifying true signals of disproportionate reporting (SDRs) and assessing the impact of factors such as sex, age, vaccine type, and state-level political inclination, this study sought to enhance understanding of vaccine safety monitoring. Additionally, by investigating the potential role of notoriety bias in the increased reporting of gustatory AEs, the findings may help refine pharmacovigilance methodologies and improve risk communication strategies in future vaccination campaigns.

Material and methods

Study design

A disproportionality analysis was conducted in October 2024 using individual case safety reports (ICSRs) from VAERS. The study focused on oral AEs reported after COVID-19 vaccination to identify SDRs and validate them using an advanced statistical approach.

The analysis was performed and reported following the REporting of A Disproportionality analysis for drUg Safety signal detection using individual case safety reports in PharmacoVigilance (READUS-PV) guidelines to ensure transparency, reproducibility and methodological rigor.[18]

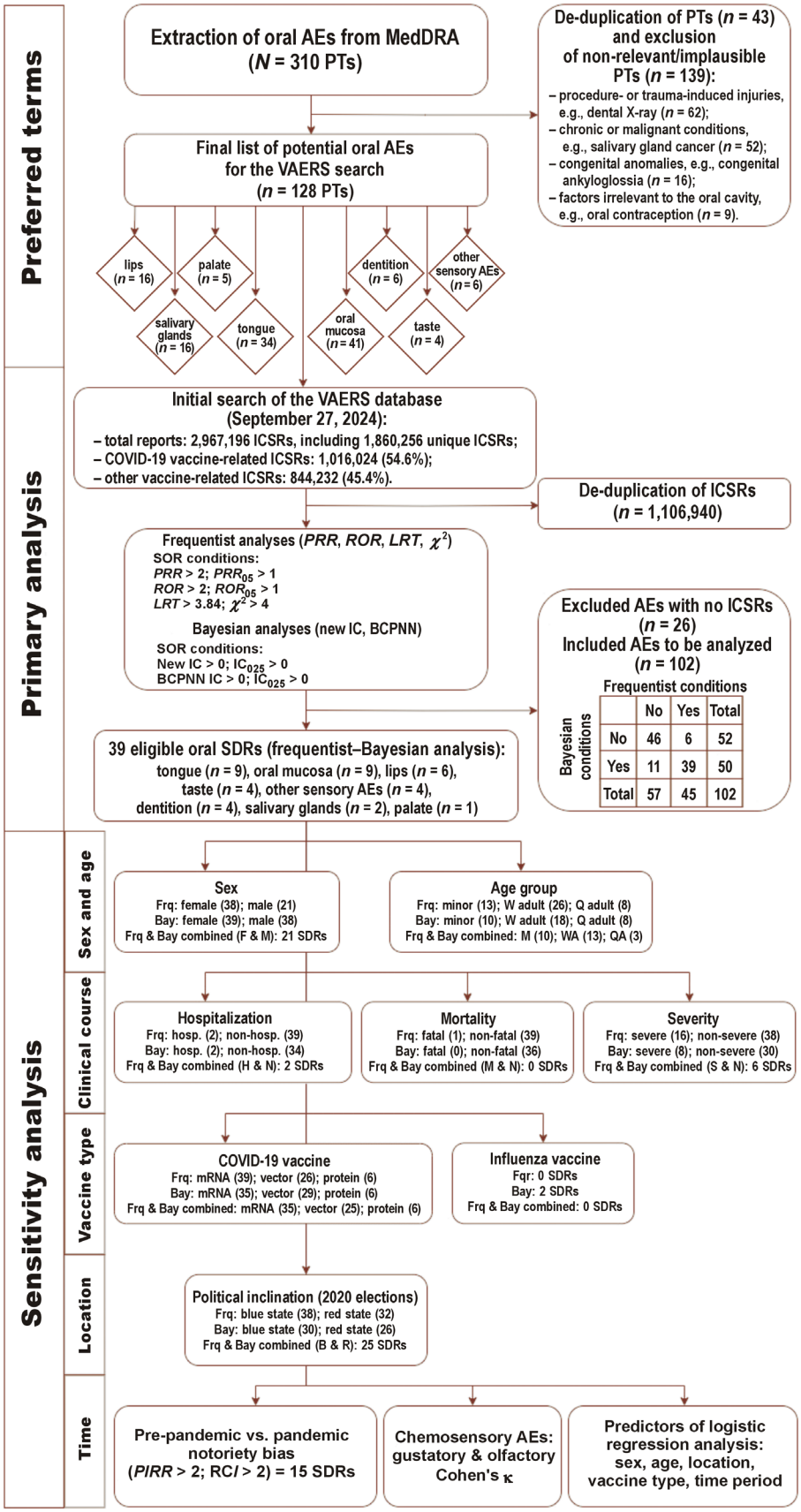

Fig. 1. Study workflow for the disproportionality analysis of oral adverse events (AEs) following coronavirus disease 2019 (COVID-19) vaccination in the United States

Bay – Bayesian; BCPNN – Bayesian Confidence Propagation Neural Network; Frq – frequentist; hosp. – hospitalized; IC – information component; ICSR – individual case safety report; *LRT* – likelihood ratio test; MedDRA – Medical Dictionary for Regulatory Activities; O adults – older adults (>64 years); *PIRR* – pandemic induced reporting ratio; *PRR* – proportional reporting ratio; PT – preferred term; *RCI* – reporting change index; *ROR* – reporting odds ratio; SDR – signal of disproportionate reporting; VAERS – Vaccine Adverse Event Reporting System; W adult – working-age adults (18–64 years).

Data source

The Vaccine Adverse Event Reporting System is a passive surveillance database jointly managed by the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA). It collects AE reports from healthcare professionals, vaccine manufacturers and the general public.[19] The Medical Dictionary for Regulatory Activities (MedDRA) is a hierarchical coding system used by VAERS to classify AEs for efficient data retrieval.[20]

The dataset was downloaded from the official VAERS website in October 2024 and included all ICSRs submitted between January 1990 and September 2024. The raw dataset comprised 2,967,196 ICSRs. After removing 1,106,940 duplicate records, 1,860,256 unique ICSRs remained. Of these, 1,016,024 (54.6%) were related to COVID-19 vaccines, and 844,232 (45.4%) were associated with other vaccines (Fig. 1).

Variables

To analyze oral AEs, an anatomophysiological framework was applied, categorizing AEs by anatomical regions (lips, palate, tongue, dentition, salivary glands, and oral mucosa) and functions (taste and other sensations). A MedDRA search at the preferred term (PT) level identified 128 PTs relevant to the oral cavity. This framework was described in a previous study[7] and is presented in Fig. 1.[7]

Oral AE reports were stratified by sex (female/male), age group (infants, children, young adults, middle-aged adults, pre-seniors, seniors, and elderly) according to the United States Census Bureau classification,[21] clinical course (severe, hospitalized or fatal) according to the definitions provided in the VAERS user guidelines,[6] vaccine type (mRNA, vector-based and protein subunit), and state-level political inclination (blue (Democratic) or red (Republican)) as indicated by the outcomes of the US 2020 presidential election.[22] Lastly, to assess temporal trends, the study period was divided into pre-pandemic (1990–2019) and pandemic (2020–2024) phases.

Statistical analysis

Statistical analyses were conducted in 2 stages. The primary analysis assessed disproportionate reporting of 128 potential oral AEs to identify true SDRs by applying both the frequentist and Bayesian conditions. This resulted in the identification of 39 oral AEs as true SDRs, as they met the predefined signal detection criteria.

The frequentist criteria for identifying true SDRs were as follows:

– proportional reporting ratio (*PRR*) ≥2, with the lower bound of the 95% confidence interval (95% *CI*) ≥1;
– reporting odds ratio (*ROR*) ≥2, with the lower bound of the 95% *CI* ≥ 1;
– likelihood ratio test (*LRT*) ≥3.84;
– χ^2 test with Yates's correction ≥4;
– total number of reports >3.

The Bayesian criteria were:

– new information component (IC) method, requiring both the point estimate and the lower limit of the 95% *CI* for the IC (IC_{025}) to be >0;
– Bayesian Confidence Propagation Neural Network (BCPNN) method, requiring both the point estimate and the IC_{025} to be >0.

A secondary (sensitivity) analysis was conducted on the 39 true SDRs to control for potential reporting biases, including selective reporting bias and notoriety bias. Disproportionality estimates were stratified by sex, age, clinical severity, vaccine type, vaccine schedule, state-level political inclination, and time period.

To examine potential notoriety bias in gustatory AEs, Cohen's kappa coefficient was calculated to assess the agreement between gustatory and olfactory AEs, stratified by time period, sex, age, and vaccine type. Specifically, agreement coefficients were compared between pre-pandemic and pandemic periods, as well as between COVID-19 and non-COVID-19 vaccines during the pandemic. Multivariable logistic regression analyses were conducted to estimate adjusted odds ratios (*AOR*s) for true SDRs, adjusting for sex, age, state-level political inclination, vaccine type, and time period.

All statistical analyses were conducted using the R software, v. 4.4.2 (https://www.r-project.org).[23]

Results

As of September 2024, VAERS contained 1,860,256 unique ICSRs, of which 56,501 reported oral AEs (30.37 cases per 1,000 ICSRs). The crude *PRR* of oral AEs following COVID-19 vaccination was 2.40 (95% *CI*: 2.35–2.44), indicating a higher reporting frequency compared with other vaccines. Females exhibited a higher *PRR* (2.29) than males (1.67), and younger vaccine recipients had lower *PRR*s than older groups. Non-severe, non-hospitalized and non-fatal cases showed higher *PRR*s than severe, hospitalized and fatal cases. Proportional reporting ratios were nearly identical in red and blue states (Table 1).

Primary analysis

Of the 128 oral AEs evaluated, 45 met the frequentist criteria, 50 met the Bayesian criteria, and 39 satisfied both, thereby qualifying as true SDRs. These SDRs were distributed across tongue (n = 9), oral mucosa (n = 9), lips (n = 6), taste (n = 4), other sensations (n = 4), dentition (n = 4), salivary glands (n = 2), and palate (n = 1).

Within taste-related AEs, taste disorder showed the highest *PRR* (23.96), followed by ageusia (17.79), hypogeusia (6.25) and dysgeusia (3.22). Other sensory AEs also

Table 1. Frequency and reporting ratios of oral adverse events (AEs) across demographic and clinical subgroups in the United States

Variable		ICSRs, *n* (%)	Oral AEs, *n* (rate per 1,000 ICSRs)	Frequentist			Bayesian
				PRR (95% *CI*)	*ROR* (95% *CI*)	χ^2 test statistic	IC (lower bound of the 95% *CI*)
Sex	female	1,102,436 (59.26)	42,740 (38.77)	2.29 (2.24–2.35)	2.36 (2.31–2.42)	5,830.65	1.15 (1.08)
	male	594,129 (31.94)	12,541 (21.11)	1.67 (1.61–1.73)	1.69 (1.63–1.75)	764.71	1.15 (0.98)
	unknown	163,691 (8.80)	1,220 (7.45)	6.21 (5.50–7.00)	6.31 (5.59–7.13)	1,148.69	3.30 (2.36)
Age group	infants	149,920 (8.06)	1,238 (8.26)	0.78 (0.56–1.10)	0.78 (0.55–1.10)	1.96	3.81 (2.79)
	children	185,715 (9.98)	2,292 (12.34)	0.89 (0.81–0.98)	0.89 (0.81–0.98)	5.72	1.80 (1.26)
	young adults	252,743 (13.59)	8,054 (31.87)	1.64 (1.57–1.72)	1.67 (1.59–1.75)	417.43	0.94 (0.76)
	middle-aged adults	276,014 (14.84)	13,884 (50.30)	1.59 (1.52–1.65)	1.62 (1.55–1.69)	466.80	0.58 (0.47)
	pre-seniors	332,348 (17.87)	15,557 (46.81)	1.77 (1.70–1.84)	1.81 (1.74–1.89)	878.99	0.74 (0.64)
	seniors	309,914 (16.66)	10,106 (32.61)	1.66 (1.59–1.74)	1.69 (1.61–1.77)	497.84	0.81 (0.65)
	elderly	86,378 (4.64)	1,650 (19.10)	1.12 (1.00–1.25)	1.12 (1.00–1.25)	3.57	0.47 (–0.04)
	unknown	267,224 (14.36)	3,720 (13.92)	4.52 (4.20–4.86)	4.61 (4.29–4.97)	1,980.13	2.29 (1.90)
Severity	severe	320,852 (17.25)	7,629 (23.78)	0.79 (0.75–0.83)	0.78 (0.74–0.82)	92.15	1.35 (1.13)
	non-severe	1,539,404 (82.75)	48,872 (31.75)	3.04 (2.97–3.11)	3.14 (3.06–3.21)	10,448.42	1.22 (1.15)
Mortality	fatal	24,675 (1.33)	167 (6.77)	0.93 (0.65–1.32)	0.93 (0.65–1.32)	0.07	0.34 (–2.28)
	non-fatal	1,835,581 (98.67)	56,334 (30.69)	2.42 (2.38–2.47)	2.49 (2.44–2.53)	9301.76	1.33 (1.26)
Hospitalization	hospitalized	131,117 (7.05)	2,751 (20.98)	0.73 (0.68–0.79)	0.73 (0.67–0.79)	64.05	0.38 (0.01)
	not hospitalized	1,729,139 (92.95)	53,750 (31.08)	2.59 (2.54–2.64)	2.66 (2.61–2.71)	10,161.07	1.39 (1.32)
ER visit	visited ER	186,794 (10.04)	4,868 (26.06)	2.50 (1.35–4.63)	2.60 (1.35–5.03)	6.06	3.39 (–4.92)
	did not visit ER	1,673,462 (89.96)	51,633 (30.85)	2.80 (2.74–2.86)	2.88 (2.82–2.94)	9,408.82	1.14 (1.07)
Life threat	life-threatening	25,714 (1.38)	1,135 (44.14)	0.92 (0.82–1.03)	0.91 (0.81–1.03)	2.10	0.70 (0.29)
	non-life-threatening	1,834,542 (98.62)	55,366 (30.18)	2.45 (2.40–2.49)	2.51 (2.46–2.56)	9,251.78	1.33 (1.26)
Political inclination	red state	597,166 (32.10)	19,189 (32.13)	2.41 (2.33–2.48)	2.47 (2.39–2.55)	3,229.49	1.39 (1.28)
	blue state	969,667 (52.13)	31,424 (32.41)	2.40 (2.34–2.46)	2.46 (2.40–2.53)	5,107.13	1.33 (1.24)
	other (not specified)	293,423 (15.77)	5,888 (20.07)	2.80 (2.63–2.99)	2.85 (2.67–3.05)	1,072.24	1.15 (0.89)
Total		1,860,256 (100.00)	56,501 (30.37)	2.40 (2.35–2.44)	2.46 (2.41–2.50)	9,058.75	1.32 (1.25)

The Bayesian Confidence Propagation Neural Network (BCPNN) method was used to calculate the information component (IC) and its lower bound of the 95% confidence interval (95% *CI*).
ER – emergency room; ICSR – individual case safety report; *PRR* – proportional reporting ratio; *ROR* – reporting odds ratio.

exhibited high *PRR*s, including oral anesthesia (16.62), burning mouth syndrome (4.67), oral paresthesia (3.72), and oral hypoesthesia (3.50).

For oral mucosal AEs, aphthous ulcer (4.43), oral lichen planus (3.12) and oral pruritus (2.65) were identified as true SDRs. In contrast, oral herpes (1.51) and oral candidiasis (1.39) did not meet the criteria for true SDRs (Table 2).

Sensitivity analyses

Sex

The frequentist criteria identified 38 true SDRs in females and 21 in males, while the Bayesian criteria identified 39 true SDRs in females and 38 in males. Twenty-one SDRs were confirmed by both methods across both sexes.

Taste disorder had the highest *PRR* in both females (19.23) and males (28.06). In females, this was followed by dental paresthesia (18.30) and ageusia (15.97), while in males, tongue erythema (23.04) and ageusia (17.25) were the most prominent (Supplementary File: Table S1 (https://doi.org/10.17605/OSF.IO/3S8KW)).

Age

Infants and toddlers (0–4 years) showed only 1 true SDR (oral hypoesthesia (*PRR*: 26.77)), while children and adolescents (5–17 years) had 4 true SDRs, namely taste disorder (20.71), ageusia (11.10), aphthous ulcer (9.53), and mouth swelling (3.71).

Young adults (18–34 years) exhibited 6 true SDRs: taste disorder (27.72); ageusia (11.34); tongue discomfort (5.87); tongue pruritus (5.66); mouth swelling (2.83); and oral paresthesia (2.14). Similarly, middle-aged adults (35–49 years) showed 7 true SDRs, such as taste disorder (14.46), ageusia (9.50), tongue discomfort (5.48), tongue pruritus (4.82), mouth swelling (4.37), toothache (3.05),

Table 2. Frequentist and Bayesian analyses of oral adverse events (AEs) reported following coronavirus disease 2019 (COVID-19) vaccination in the United States (as of September 2024)

AE category	Preferred term (MedDRA number)	*ARR* (per 1,000 reports following COVID-19 vaccination)	Frequentist	
			PRR (95% *CI*)	*ROR* (95% *CI*)
Taste	ageusia (10001480)	6.11	17.79 (15.81–20.01)	17.89 (15.91–20.13)
	dysgeusia (10013911)	5.07	3.22 (3.03–3.42)	3.23 (3.04–3.44)
	hypogeusia (10020989)	0.19	6.25 (4.12–9.48)	6.25 (4.12–9.49)
	taste disorder (10082490)	2.58	23.96 (19.44–29.53)	24.02 (19.49–29.60)
Other sensory AEs	anesthesia oral (10082548)	0.02	16.62 (2.23–123.83)	16.62 (2.23–123.83)
	burn oral cavity (10075532)	0.01	1.87 (0.58–6.07)	1.87 (0.58–6.07)
	burning mouth syndrome (10068065)	0.04	4.67 (2.20–9.91)	4.67 (2.20–9.92)
	hypoesthesia oral (10057371)	4.97	3.50 (3.29–3.73)	3.51 (3.30–3.74)
	oral dysesthesia (10050820)	0.004	1.11 (0.25–4.95)	1.11 (0.25–4.95)
	paresthesia oral (10057372)	6.72	3.72 (3.52–3.93)	3.73 (3.53–3.95)
Dentition[1]	dental discomfort (10054217)	0.07	15.16 (5.54–41.49)	15.17 (5.54–41.49)
	dental paresthesia (10078276)	0.03	12.88 (3.08–53.81)	12.88 (3.08–53.82)
	hyperesthesia teeth (10082426)	0.18	11.76 (6.70–20.64)	11.76 (6.70–20.64)
	hypoesthesia teeth (10051780)	0.01	5.82 (1.32–25.59)	5.82 (1.32–25.59)
	toothache (10044055)	1.19	3.23 (2.86–3.66)	3.24 (2.86–3.66)
Salivary glands and saliva[2]	aptyalism (10003068)	0.04	4.63 (2.07–10.35)	4.63 (2.07–10.35)
	dry mouth (10013781)	2.43	2.65 (2.44–2.87)	2.65 (2.44–2.87)
	non-infective sialoadenitis (10075243)	0.01	3.12 (1.03–9.39)	3.12 (1.03–9.39)
	saliva altered (10039379)	0.02	1.74 (0.85–3.56)	1.74 (0.85–3.56)
	saliva discoloration (10049069)	0.01	1.16 (0.37–3.67)	1.16 (0.37–3.67)
	salivary gland calculus (10039394)	0.00	3.32 (0.37–29.74)	3.32 (0.37–29.74)
	salivary gland disorder (10061935)	0.02	3.32 (1.11–9.94)	3.32 (1.11–9.94)
	salivary gland enlargement (10039408)	0.05	0.70 (0.49–1.01)	0.70 (0.49–1.01)
	salivary gland pain (10039421)	0.03	1.50 (0.80–2.81)	1.50 (0.80–2.81)
	salivary hypersecretion (10039424)	0.29	0.51 (0.44–0.59)	0.51 (0.44–0.59)
	sialoadenitis (10040628)	0.03	0.14 (0.10–0.20)	0.14 (0.10–0.20)
Tongue[3]	glossitis (10018386)	0.16	1.44 (1.11–1.87)	1.44 (1.11–1.87)
	glossodynia (10018388)	0.63	2.22 (1.92–2.58)	2.22 (1.92–2.58)
	hypertrophy of tongue papillae (10020893)	0.01	3.05 (0.85–10.92)	3.05 (0.85–10.92)
	macroglossia (10025391)	0.001	1.66 (0.15–18.33)	1.66 (0.15–18.33)
	plicated tongue (10035630)	0.01	1.83 (0.64–5.26)	1.83 (0.64–5.26)
	strawberry tongue (10051495)	0.01	0.64 (0.28–1.46)	0.64 (0.28–1.46)
	stiff tongue (10081491)	0.01	9.14 (1.18–70.80)	9.14 (1.18–70.80)
	swollen tongue (10042727)	4.68	2.36 (2.23–2.50)	2.37 (2.24–2.50)
	tongue blistering (10043942)	0.17	2.75 (2.01–3.77)	2.75 (2.01–3.77)
	tongue coated (10043945)	0.05	1.90 (1.14–3.17)	1.90 (1.14–3.17)
	tongue discoloration (10043949)	0.21	1.64 (1.30–2.06)	1.64 (1.30–2.06)
	tongue disorder (10043951)	0.58	1.09 (0.97–1.24)	1.10 (0.97–1.24)
	tongue discomfort (10077855)	0.65	9.06 (6.97–11.77)	9.06 (6.97–11.78)
	tongue dry (10049713)	0.09	4.20 (2.57–6.87)	4.20 (2.57–6.87)
	tongue eruption (10052002)	0.08	2.98 (1.89–4.68)	2.98 (1.89–4.68)
	tongue erythema (10079075)	0.15	7.89 (4.72–13.21)	7.89 (4.72–13.21)
	tongue exfoliation (10064488)	0.01	1.31 (0.51–3.37)	1.31 (0.51–3.37)
	tongue movement disturbance (10043963)	0.06	2.88 (1.68–4.95)	2.88 (1.68–4.95)
	tongue edema (10043967)	0.04	0.07 (0.05–0.10)	0.07 (0.05–0.10)
	tongue paralysis (10043972)	0.03	0.80 (0.49–1.33)	0.80 (0.49–1.33)
	tongue pigmentation (10069164)	0.004	3.32 (0.37–29.74)	3.32 (0.37–29.74)
	tongue pruritus (10070072)	0.50	6.62 (5.11–8.59)	6.62 (5.11–8.59)
	tongue rough (10043977)	0.01	5.82 (1.32–25.59)	5.82 (1.32–25.59)
	tongue spasm (10043981)	0.01	1.78 (0.73–4.37)	1.78 (0.73–4.37)
	tongue thrust (10082545)	0.002	0.83 (0.12–5.90)	0.83 (0.12–5.90)
	tongue ulceration (10043991)	0.15	1.76 (1.32–2.33)	1.76 (1.32–2.33)
	trichoglossia (10080276)	0.01	8.31 (1.06–64.91)	8.31 (1.06–64.91)

Frequentist			Bayesian			SDR (frequentist and Bayesian)
LRT	χ^2	SDR	new IC (IC_{025})	BCPNN (IC_{025})	SDR	
5,613.85	4,404.14	yes	0.81 (1.71)	0.81 (0.77)	yes	yes
1,763.76	1,624.72	yes	0.54 (1.42)	0.54 (0.51)	yes	yes
112.86	95.94	yes	0.69 (1.39)	0.69 (0.50)	yes	yes
2,523.77	1,936.15	yes	0.82 (1.70)	0.82 (0.77)	yes	yes
17.73	12.39	yes	0.78 (1.05)	0.80 (0.19)	yes	yes
1.16	0.61	no	0.32 (0.59)	0.34 (–0.51)	no	no
22.09	18.41	yes	0.63 (1.13)	0.64 (0.24)	yes	yes
1,896.22	1,732.77	yes	0.57 (1.44)	0.57 (0.53)	yes	yes
0.02	0.00	no	0.06 (0.31)	0.07 (–1.16)	no	no
2,735.32	2,485.39	yes	0.58 (1.46)	0.58 (0.55)	yes	yes
63.18	48.57	yes	0.79 (1.36)	0.80 (0.48)	yes	yes
25.57	19.03	yes	0.77 (1.16)	0.78 (0.29)	yes	yes
147.33	118.00	yes	0.77 (1.47)	0.77 (0.57)	yes	yes
8.04	5.72	yes	0.65 (0.87)	0.68 (–0.04)	no	no
415.90	382.15	yes	0.54 (1.38)	0.54 (0.47)	yes	yes
19.00	15.69	yes	0.62 (1.10)	0.63 (0.21)	yes	yes
642.99	602.86	yes	0.48 (1.34)	0.48 (0.43)	yes	yes
4.91	3.61	no	0.51 (0.81)	0.53 (–0.15)	no	no
2.40	1.83	no	0.30 (0.79)	0.31 (–0.22)	no	no
0.07	0.00	no	0.09 (0.44)	0.09 (–0.84)	no	no
1.41	0.48	no	0.48 (0.42)	0.55 (–0.76)	no	no
5.66	4.23	yes	0.53 (0.83)	0.55 (–0.11)	no	no
3.71	3.38	no	–0.26 (0.63)	–0.26 (–0.58)	no	no
1.61	1.22	no	0.23 (0.78)	0.24 (–0.25)	no	no
88.87	88.37	no	–0.53 (0.62)	–0.53 (–0.66)	no	no
165.73	152.35	no	–1.91 (0.19)	–1.92 (–2.28)	no	no
7.98	7.49	no	0.22 (0.99)	0.22 (0.02)	yes	no
123.31	116.82	yes	0.41 (1.23)	0.41 (0.31)	yes	yes
3.50	2.35	no	0.50 (0.72)	0.52 (–0.27)	no	no
0.18	0.00	no	0.23 (0.19)	0.29 (–1.50)	no	no
1.33	0.78	no	0.32 (0.63)	0.33 (–0.44)	no	no
1.15	0.75	no	–0.31 (0.39)	–0.33 (–1.07)	no	no
8.00	5.24	yes	0.71 (0.83)	0.75 (–0.07)	no	no
1,025.91	972.37	yes	0.44 (1.32)	0.44 (0.40)	yes	yes
46.77	42.86	yes	0.49 (1.20)	0.49 (0.29)	yes	yes
6.44	5.63	no	0.34 (0.94)	0.35 (–0.02)	no	no
18.47	17.55	no	0.28 (1.06)	0.28 (0.11)	yes	no
2.11	2.02	no	0.06 (0.96)	0.06 (–0.04)	no	no
482.07	399.22	yes	0.75 (1.55)	0.75 (0.64)	yes	yes
43.06	37.49	yes	0.61 (1.24)	0.61 (0.34)	yes	yes
26.54	23.70	yes	0.51 (1.14)	0.52 (0.24)	yes	yes
103.48	85.72	yes	0.73 (1.40)	0.73 (0.51)	yes	yes
0.31	0.10	no	0.15 (0.57)	0.16 (–0.59)	no	no
17.44	15.33	yes	0.50 (1.08)	0.51 (0.17)	yes	yes
528.48	464.43	no	–2.77 (0.11)	–2.78 (–3.10)	no	no
0.72	0.53	no	–0.15 (0.61)	–0.15 (–0.59)	no	no
1.41	0.48	no	0.48 (0.42)	0.55 (–0.76)	no	no
316.74	270.08	yes	0.70 (1.49)	0.70 (0.58)	yes	yes
8.04	5.72	yes	0.65 (0.87)	0.68 (–0.04)	no	no
1.68	1.13	no	0.31 (0.70)	0.32 (–0.34)	no	no
0.03	0.00	no	–0.10 (0.15)	–0.13 (–1.82)	no	no
16.14	15.14	no	0.31 (1.05)	0.31 (0.11)	yes	no
6.97	4.47	yes	0.69 (0.79)	0.74 (–0.12)	no	no

AE category	Preferred term (MedDRA number)	*ARR* (per 1,000 reports following COVID-19 vaccination)	Frequentist	
			PRR (95% *CI*)	*ROR* (95% *CI*)
Lips[4]	angular cheilitis (10002509)	0.02	3.05 (1.24–7.51)	3.05 (1.24–7.51)
	cheilitis (10008417)	0.33	1.40 (1.17–1.67)	1.40 (1.17–1.67)
	chapped lips (10049047)	0.25	2.06 (1.64–2.60)	2.07 (1.64–2.60)
	lip blister (10049307)	0.32	1.43 (1.20–1.72)	1.43 (1.20–1.72)
	lip discoloration (10024549)	0.12	1.37 (1.02–1.84)	1.37 (1.02–1.84)
	lip disorder (10048470)	0.14	3.65 (2.50–5.33)	3.65 (2.50–5.33)
	lip dry (10024552)	0.22	2.38 (1.84–3.07)	2.38 (1.84–3.07)
	lip erythema (10080124)	0.17	3.25 (2.32–4.54)	3.25 (2.32–4.54)
	lip exfoliation (10064482)	0.07	1.35 (0.93–1.98)	1.35 (0.93–1.98)
	lip edema (10024558)	0.06	0.86 (0.61–1.21)	0.86 (0.61–1.21)
	lip pain (10024561)	0.30	2.44 (1.95–3.06)	2.44 (1.95–3.06)
	lip pruritus (10070721)	0.39	4.17 (3.27–5.30)	4.17 (3.27–5.30)
	lip scab (10082767)	0.01	5.82 (0.72–47.28)	5.82 (0.72–47.28)
	lip swelling (10024570)	5.64	1.56 (1.50–1.63)	1.57 (1.50–1.64)
	lip ulceration (10024572)	0.03	0.92 (0.55–1.53)	0.92 (0.55–1.53)
Palate[5]	palatal disorder (10052453)	0.02	1.22 (0.63–2.35)	1.22 (0.63–2.35)
	palatal edema (10056998)	0.01	0.65 (0.32–1.30)	0.65 (0.32–1.30)
	palatal swelling (10074403)	0.09	4.35 (2.59–7.31)	4.35 (2.59–7.31)
	palatal ulcer (10077519)	0.004	1.66 (0.30–9.07)	1.66 (0.30–9.07)
Oral mucosa[6]	aphthous ulcer (10002959)	0.40	4.43 (3.47–5.66)	4.43 (3.47–5.66)
	circumoral edema (10052250)	0.01	1.83 (0.64–5.26)	1.83 (0.64–5.26)
	circumoral swelling (10081703)	0.05	4.71 (2.32–9.56)	4.71 (2.32–9.56)
	coating in the mouth (10075366)	0.02	4.15 (1.42–12.16)	4.15 (1.42–12.16)
	leukoplakia oral (10024396)	0.01	0.48 (0.19–1.23)	0.48 (0.19–1.23)
	mouth swelling (10075203)	1.03	3.51 (3.05–4.03)	3.51 (3.06–4.03)
	mouth ulceration (10028034)	0.42	0.75 (0.65–0.85)	0.75 (0.65–0.85)
	edema mouth (10030110)	0.01	0.05 (0.03–0.09)	0.05 (0.03–0.09)
	oral blood blister (10076590)	0.05	3.91 (1.97–7.73)	3.91 (1.97–7.73)
	oral candidiasis (10030963)	0.14	1.39 (1.06–1.82)	1.39 (1.06–1.82)
	oral discomfort (10030973)	0.86	3.38 (2.91–3.93)	3.38 (2.91–3.93)
	oral disorder (10067621)	0.22	1.47 (1.18–1.83)	1.47 (1.18–1.83)
	oral fungal infection (10061324)	0.02	1.66 (0.71–3.88)	1.66 (0.71–3.88)
	oral herpes (10067152)	1.52	1.51 (1.39–1.64)	1.51 (1.39–1.64)
	oral lichen planus (10030983)	0.04	3.12 (1.65–5.89)	3.12 (1.65–5.89)
	oral mucosa erosion (10064594)	0.001	0.83 (0.05–13.28)	0.83 (0.05–13.28)
	oral mucosal blistering (10030995)	0.43	1.46 (1.25–1.71)	1.46 (1.25–1.71)
	oral mucosal discoloration (10030996)	0.01	0.91 (0.39–2.15)	0.91 (0.39–2.15)
	oral mucosal eruption (10030997)	0.20	1.33 (1.06–1.66)	1.33 (1.06–1.66)
	oral mucosal erythema (10067418)	0.09	1.61 (1.14–2.28)	1.61 (1.14–2.28)
	oral mucosal exfoliation (10064487)	0.03	1.34 (0.74–2.41)	1.34 (0.74–2.41)
	oral pain (10031009)	0.82	2.21 (1.94–2.52)	2.21 (1.94–2.52)
	oral papule (10031010)	0.002	0.55 (0.09–3.32)	0.55 (0.09–3.32)
	oral pruritus (10052894)	0.50	2.65 (2.22–3.17)	2.65 (2.22–3.17)
	oral purpura (10083533)	0.003	2.49 (0.26–23.97)	2.49 (0.26–23.97)
	oral pustule (10056674)	0.01	0.83 (0.40–1.74)	0.83 (0.40–1.74)
	oropharyngeal blistering (10067950)	0.06	0.60 (0.42–0.84)	0.60 (0.42–0.84)
	oropharyngeal plaque (10067721)	0.01	1.45 (0.43–4.97)	1.45 (0.43–4.97)
	perioral dermatitis (10034541)	0.02	1.25 (0.60–2.59)	1.25 (0.60–2.59)
	stomatitis (10042128)	0.96	1.48 (1.33–1.64)	1.48 (1.33–1.64)

The frequentist criteria for identifying a signal of disproportionate reporting (SDR) were as follows: 1) *PRR* ≥ 2 with a 95% *CI* lower bound ≥1; 2) *ROR* ≥ 2 with a 95% *CI* lower bound ≥1; 3) likelihood ratio test (*LRT*) ≥3.84; 4) χ^2 with Yates's correction ≥4; and 5) total number of reports >3.
The Bayesian criteria for identifying an SDR were as follows: 1) point estimate and lower limit of the 95% *CI* of the new IC method (IC_{025}) are >0; and 2) Bayesian Confidence Propagation Neural Network (BCPNN) point estimate and corresponding IC_{025} are >0.
[1] The preferred term "sensitivity of teeth (10040012)" was not reported in any vaccine groups.
[2] The preferred terms "salivary duct inflammation (10056681)", "salivary duct obstruction (10039386)", "salivary duct stenosis (10039388)", "salivary gland induration (10071363)", and "salivary gland mass (10057002)" were not reported in any vaccine groups.
[3] The preferred terms "acquired macroglossia (10058835)", "ankyloglossia acquired (10049243)", "atrophic glossitis (10069085)", "atrophy of tongue papillae (10003712)", "tongue black hairy (10043941)", "tongue fungal infection (10075845)", and "tongue induration (10084548)" were not reported in any vaccine groups.

Frequentist			Bayesian			SDR (frequentist and Bayesian)
LRT	χ^2	SDR	new IC (IC_{025})	BCPNN (IC_{025})	SDR	
7.00	5.55	yes	0.51 (0.90)	0.52 (–0.03)	no	no
14.25	13.72	no	0.20 (1.03)	0.20 (0.06)	yes	no
41.25	38.89	yes	0.38 (1.15)	0.38 (0.22)	yes	yes
16.09	15.48	no	0.21 (1.04)	0.21 (0.07)	yes	no
4.46	4.10	no	0.19 (0.94)	0.19 (–0.04)	no	no
56.85	50.67	yes	0.57 (1.26)	0.58 (0.36)	yes	yes
48.57	45.25	yes	0.44 (1.18)	0.44 (0.27)	yes	yes
57.80	52.22	yes	0.54 (1.24)	0.54 (0.34)	yes	yes
2.48	2.16	no	0.18 (0.88)	0.18 (–0.12)	no	no
0.76	0.61	no	–0.10 (0.72)	–0.11 (–0.40)	no	no
68.69	64.14	yes	0.45 (1.22)	0.45 (0.30)	yes	yes
176.39	157.29	yes	0.61 (1.38)	0.61 (0.48)	yes	yes
4.02	2.29	no	0.62 (0.64)	0.68 (–0.33)	no	no
414.45	405.24	no	0.26 (1.16)	0.26 (0.22)	yes	no
0.10	0.04	no	–0.06 (0.66)	–0.06 (–0.49)	no	no
0.35	0.18	no	0.12 (0.68)	0.12 (–0.41)	no	no
1.52	1.12	no	–0.31 (0.45)	–0.32 (–0.95)	no	no
41.18	35.65	yes	0.62 (1.23)	0.62 (0.34)	yes	yes
0.36	0.03	no	0.25 (0.36)	0.29 (–0.98)	no	no
190.27	168.67	yes	0.62 (1.39)	0.62 (0.49)	yes	yes
1.33	0.78	no	0.32 (0.63)	0.33 (–0.44)	no	no
25.19	21.14	yes	0.63 (1.15)	0.64 (0.26)	yes	yes
8.89	6.87	yes	0.59 (0.93)	0.61 (0.02)	yes	yes
2.42	1.76	no	–0.54 (0.29)	–0.57 (–1.43)	no	no
393.64	358.70	yes	0.57 (1.39)	0.57 (0.48)	yes	yes
19.51	19.33	no	–0.21 (0.79)	–0.21 (–0.32)	no	no
249.85	214.24	no	–3.11 (0.06)	–3.15 (–3.69)	no	no
19.71	16.72	yes	0.59 (1.11)	0.59 (0.21)	yes	yes
5.85	5.46	no	0.20 (0.96)	0.20 (–0.01)	no	no
314.39	287.33	yes	0.56 (1.37)	0.56 (0.47)	yes	yes
12.51	11.92	no	0.23 (1.02)	0.23 (0.06)	yes	no
1.44	0.96	no	0.28 (0.70)	0.29 (–0.34)	no	no
95.20	93.00	no	0.24 (1.12)	0.24 (0.17)	yes	no
14.72	12.65	yes	0.52 (1.05)	0.53 (0.14)	yes	yes
0.02	0.00	no	–0.09 (0.07)	–0.13 (–2.53)	no	no
23.39	22.62	no	0.22 (1.06)	0.22 (0.10)	yes	no
0.04	0.00	no	–0.06 (0.49)	–0.06 (–0.79)	no	no
6.36	6.01	no	0.17 (0.98)	0.17 (0.00)	no	no
7.49	6.87	no	0.27 (0.97)	0.27 (0.01)	yes	no
0.96	0.69	no	0.17 (0.76)	0.18 (-0.29)	no	no
158.52	150.47	yes	0.41 (1.24)	0.41 (0.32)	yes	yes
0.43	0.04	no	–0.37 (0.13)	–0.45 (–2.09)	no	no
132.47	123.48	yes	0.48 (1.27)	0.48 (0.36)	yes	yes
0.71	0.10	no	0.38 (0.31)	0.46 (–1.04)	no	no
0.24	0.09	no	–0.12 (0.51)	–0.13 (–0.77)	no	no
8.85	8.39	no	–0.38 (0.58)	–0.39 (–0.70)	no	no
0.37	0.09	no	0.20 (0.48)	0.22 (–0.73)	no	no
0.35	0.17	no	0.13 (0.65)	0.14 (–0.45)	no	no
55.21	53.86	no	0.23 (1.10)	0.23 (0.15)	yes	no

[4] The preferred term "lip erosion (10051992)" was not reported in any vaccine groups.
[5] The preferred term "palatal palsy (10072012)" was not reported in any vaccine groups.
[6] The preferred terms "aphthous stomatitis (10002958)", "buccal mucosal roughening (10048479)", "mouth plaque (10028032)", "oral lichenoid reaction (10083833)", "oral mucosal hypertrophy (10062956)", "oral mucosal petechiae (10030998)", "oral mucosal roughening (10084009)", "oral mucosal scab (10082769)", "oral pigmentation (10077552)", "oral soft tissue disorder (10061326)", and "oral viral infection (10065234)" were not reported in any vaccine groups.
ARR – absolute reporting ratio; MedDRA – Medical Dictionary for Regulatory Activities.

and oral paresthesia (2.08). Moreover, pre-seniors (50–64 years) had 8 true SDRs: taste disorder (12.86); ageusia (11.72); tongue discomfort (5.46); tongue pruritus (5.19); oral paresthesia (2.34); dysgeusia (2.31); oral hypoesthesia (2.21); and oral discomfort (2.03).

Seniors (65–79 years) had 4 true SDRs, namely taste disorder (11.52), ageusia (10.43), tongue discomfort (4.99), and oral paresthesia (2.13), whereas ageusia (7.54) was the only true SDR identified among individuals aged ≥80 years (Supplementary File: Table S2).

Clinical course

Severe cases had 8 true SDRs, while non-severe cases showed 30 true SDRs. Six SDRs were shared between both groups, including ageusia (severe (*PRR*: 10.04) vs. non-severe *PRR*: 22.38)), hypogeusia (6.81 vs. 6.49), taste disorder (31.94 vs. 21.12), tongue discomfort (5.12 vs. 8.81), tongue pruritus (3.61 vs. 6.59), and mouth swelling (2.28 vs. 3.48) (Supplementary File: Table S3).

Hospitalized cases showed only 2 true SDRs, compared with 34 among non-hospitalized cases. Both hospitalized and non-hospitalized cases had jointly 2 true SDRs, including ageusia (hospitalized (*PRR*: 11.73) vs. non-hospitalized (*PRR*: 18.30)) and taste disorder (9.65 vs. 25.40, respectively) (Supplementary File: Table S4).

Mortality cases showed no true SDRs, while non-mortality cases exhibited 36 true SDRs. Among fatal cases, the Bayesian criteria were not met by any oral AE, while the frequentist conditions were met by ageusia only (*PRR*: 7.81) (Supplementary File: Table S5).

Vaccine schedule

Primary COVID-19 vaccination doses were associated with 35 true SDRs, while booster doses showed 10 true SDRs. Nine SDRs were shared between both schedules, including ageusia (primer (*PRR*: 17.79) vs. booster (*PRR*: 17.68)), hypogeusia (6.20 vs. 7.47), taste disorder (24.37 vs. 14.14), toothache (3.21 vs. 3.79), tongue blistering (2.73 vs. 3.26), tongue erythema (7.95 vs. 6.49), lip pain (2.45 vs. 2.24), aphthous ulcer (4.45 vs. 3.82), and mouth swelling (3.51 vs. 3.43) (Supplementary File: Table S6).

Vaccine type

The mRNA-based and viral vector-based COVID-19 vaccines exhibited 35 and 25 true SDRs, respectively, whereas protein subunit vaccines showed 6 true SDRs, as follows: ageusia (*PRR*: 18.74); taste disorder (14.93); glossodynia (4.53); dry mouth (4.21); dysgeusia (4.09); and oral paresthesia (2.31).

Among mRNA-based vaccines, taste disorder had the highest *PRR* (23.77), followed by ageusia (17.58), oral anesthesia (16.13), dental discomfort (15.23), dental paresthesia (12.54), hyperesthesia of teeth (11.85), tongue discomfort (9.11), tongue erythema (8.01), tongue pruritus (6.87), and hypogeusia (6.13). Similarly, for viral vector-based vaccines, taste disorder came first (26.07), followed by oral anesthesia (22.28), ageusia (20.55), dental paresthesia (16.71), dental discomfort (13.92), and hyperesthesia of teeth (10.28). When evaluating influenza vaccines, no true SDRs were found (Supplementary File: Table S7).

State-level political inclination

Blue (Democratic) states exhibited 30 true SDRs, and red (Republican) states showed 25 true SDRs. Both types of states shared 25 true SDRs, including taste disorder (blue (*PRR*: 23.20) vs. red (*PRR*: 25.22)), ageusia (17.27 vs. 20.56), hypogeusia (9.06 vs. 4.51), dysgeusia (3.11 vs. 3.30), oral paresthesia (3.72 vs. 3.56), oral hypoesthesia (3.57 vs. 3.32), and aphthous ulcer (4.71 vs. 4.38). Five AEs were unique to blue states: dental discomfort; dry tongue; tongue eruption; lip disorder; and palatal swelling (Supplementary File: Table S8).

Co-occurrence of chemosensory AEs

Overall, gustatory and olfactory AEs co-occurred with a Cohen's kappa (κ) of 0.459, indicating moderate agreement. The strongest agreement was observed for ageusia and anosmia (κ = 0.699), followed by hypogeusia and hyposmia (κ = 0.399), and taste disorder and parosmia (κ = 0.258) (Supplementary File: Table S9).

For non-COVID-19 vaccines, a considerable difference was observed between the pre-pandemic (January 1990–December 2019) and pandemic (January 2020–October 2024) intervals. The co-occurrence of any gustatory AE and any olfactory AE was slight in the pre-pandemic period (κ = 0.115) but moderate in the pandemic period (κ = 0.500). Ageusia and anosmia exhibited fair agreement in the pre-pandemic period (κ = 0.283), but substantial agreement was noted between the two in the pandemic interval (κ = 0.721). Similarly, hypogeusia and hyposmia showed no agreement pre-pandemic (κ < 0.001), and moderate agreement in the pandemic period (κ = 0.474).

During the pandemic, the co-occurrence of chemosensory AEs was moderate for both non-COVID-19 (κ = 0.500) and COVID-19 vaccines (κ = 0.489). Ageusia and anosmia exhibited substantial agreement for non-COVID-19 (κ = 0.721) and COVID-19 vaccines (κ = 0.710). Similarly, hypogeusia and hyposmia showed moderate agreement for non-COVID-19 (κ = 0.474) and COVID-19 vaccines (κ = 0.438) (Fig. 2).

Google Trends and VAERS reports

A non-parametric correlation analysis was performed between the weekly search volume of keywords (taste loss, ageusia and dysgeusia) in Google Trends

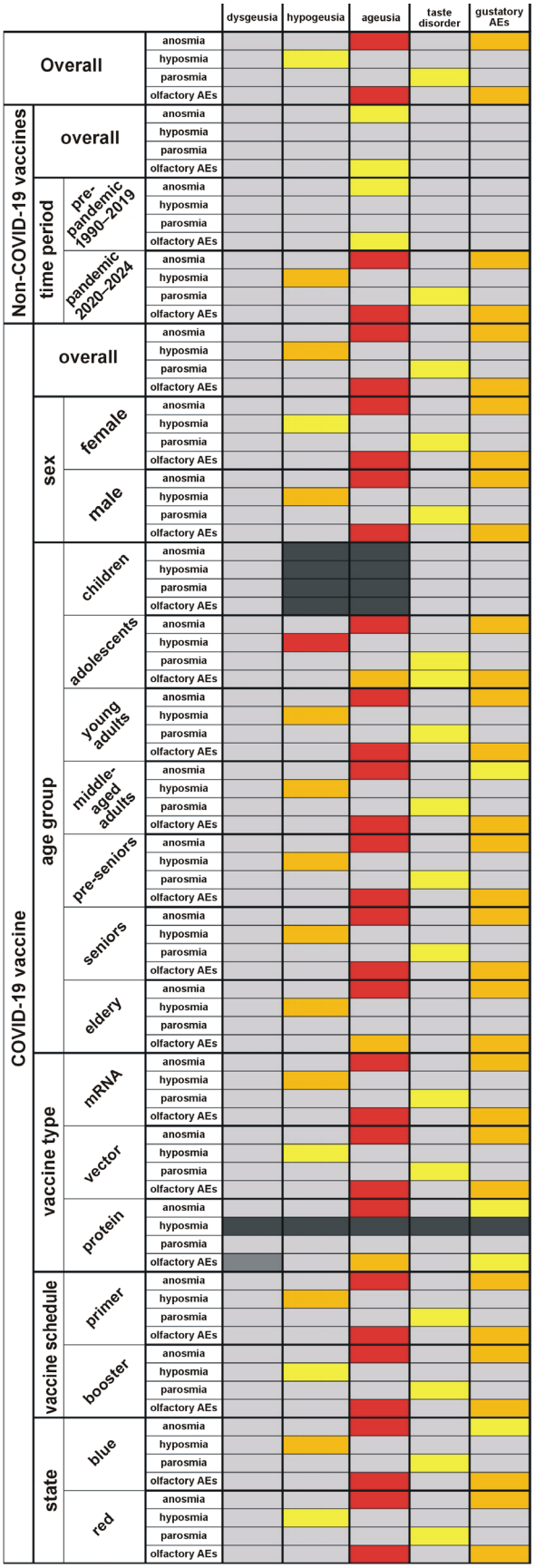

Fig. 2. Co-occurrence of chemosensory adverse events (AEs) across different subgroups

Cohen's kappa values are color-coded as follows: values <0 are shown in dark gray; 0.00–0.20 in gray; 0.21–0.40 in yellow; 0.41–0.60 in orange; and 0.61–0.80 in red. Cells with not available values due to zero reports are displayed in black.

(https://trends.google.com/trends) and the weekly number of gustatory AE reports in VAERS (taste disorder, ageusia, dysgeusia, and hypogeusia). Spearman's rho coefficient was 33.3% during the pandemic period preceding the COVID-19 vaccine rollout, and increased to 75.0% following the public administration of COVID-19 vaccines on December 14, 2020 (Fig. 3).

Regression analyses

Binary logistic regression models were constructed for each true SDR, using sex (female vs. male), age (per year increase), state (red vs. blue), and vaccine type (COVID-19 vaccines vs. non-COVID-19 vaccines) as predictors to calculate adjusted odds ratios (*AOR*s) with 95% *CI*s (Table 3).

Females had significantly higher odds for 32 SDRs, while no SDRs were more prevalent in males. Notably, elevated risks in females were observed for dysgeusia (*AOR* = 2.056 (1.921–2.199)), ageusia (*AOR* = 1.094 (1.032–1.160)), taste disorder (*AOR* = 1.464 (1.333–1.609)), oral paresthesia (*AOR* = 3.742 (3.485–4.019)), oral hypoesthesia (*AOR* = 2.433 (2.266–2.612)), dry mouth (*AOR* = 1.514 (1.387–1.653)), aphthous ulcer (*AOR* = 1.674 (1.330–2.108)), and oral lichen planus (*AOR* = 2.708 (1.211–6.055)), among others (Fig. 4A).

Increasing age was associated with higher odds for 27 SDRs. For each additional year of age, the probability of developing coating in the mouth increased by 3.4% (1.2–5.7%), burning mouth syndrome by 2.6% (1.0–4.1%), oral lichen planus by 2.4% (0.9–4.0%), and tongue erythema by 2.2% (1.3–3.0%). These findings suggest a cumulative effect of age, where the likelihood of developing these SDRs progressively increases over time (Fig. 4B).

State-level political inclination was not statistically significant for 36 SDRs. However, 3 SDRs showed significant variation: tongue blistering and ageusia were more prevalent in red states, whereas oral paresthesia was more common in blue states (Fig. 4C).

For 38 SDRs, odds were significantly higher for COVID-19 vaccines compared with other vaccines, except for coating in the mouth (Fig. 4D).

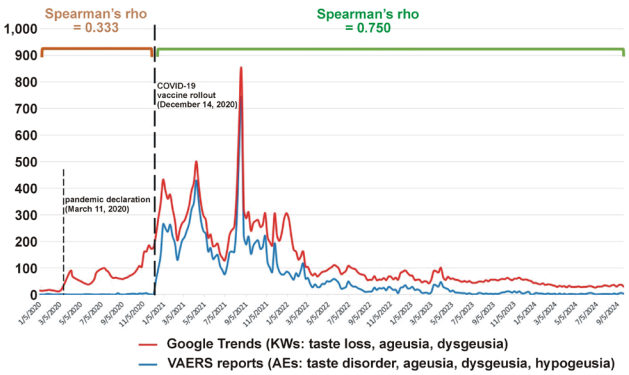

Fig. 3. Trends in taste disorder reports in the Vaccine Adverse Event Reporting System (VAERS) and Google Trends search activity (2020–2024)

KW – keyword.

Table 3. Logistic regression analysis of eligible oral signals of dipropionate reporting (SDRs) associated with coronavirus disease 2019 (COVID-19) vaccination in the United States

Preferred term	Sex (female vs. male)	Age (increase per year)	State (red vs. blue)	Vaccine type (COVID-19 vaccine vs. non-COVID-19 vaccines)
Ageusia	1.094 (1.032–1.160); 0.003*	1.007 (1.006–1.009); <0.001*	1.098 (1.040–1.160); 0.001*	15.182 (13.313–17.313); <0.001*
Dysgeusia	2.056 (1.921–2.199); <0.001*	1.007 (1.006–1.008); <0.001*	0.994 (0.941–1.050); 0.834	2.648 (2.474–2.834); <0.001*
Hypogeusia	0.784 (0.581–1.056); 0.109	1.009 (1.002–1.016); 0.011*	1.009 (0.749–1.359); 0.952	5.682 (3.528–9.152); <0.001*
Taste disorder	1.464 (1.333–1.609); <0.001*	1.008 (1.006–1.010); <0.001*	1.089 (1.002–1.183); 0.044	19.645 (15.688–24.599); <0.001*
Anesthesia oral	0.915 (0.337–2.480); 0.861	0.986 (0.963–1.009); 0.216	0.704 (0.248–2.000); 0.510	16.931 (2.111–135.767); 0.008*
Burning mouth syndrome	2.568 (1.145–5.762); 0.022*	1.026 (1.010–1.041); 0.001*	1.475 (0.816–2.665); 0.198	4.454 (1.743–11.381); 0.002*
Hypoesthesia oral	2.433 (2.266–2.612); <0.001*	1.003 (1.001–1.004); <0.001*	0.966 (0.914–1.021); 0.216	2.966 (2.768–3.179); <0.001*
Paresthesia oral	3.742 (3.485–4.019); <0.001*	1.002 (1.001–1.004); <0.001*	0.911 (0.869–0.956); <0.001*	3.164 (2.977–3.362); <0.001*
Dental discomfort	1.893 (1.029–3.480); 0.040*	1.016 (1.003–1.029); 0.015*	1.047 (0.633–1.730); 0.859	39.081 (5.394–283.152); <0.001*
Dental paresthesia	1.465 (0.581–3.694); 0.418	0.999 (0.980–1.018); 0.909	0.993 (0.434–2.270); 0.987	8.719 (1.972–38.555); 0.004*
Hyperesthesia teeth	1.806 (1.237–2.637); 0.002*	1.007 (1.000–1.015); 0.062	0.876 (0.633–1.211); 0.423	10.397 (5.433–19.895); <0.001*
Toothache	1.564 (1.376–1.778); <0.001*	1.016 (1.014–1.019); <0.001*	0.926 (0.827–1.036); 0.180	2.351 (2.051–2.695); <0.001*
Aptyalism	0.970 (0.510–1.842); 0.925	1.018 (1.003–1.033); 0.018*	1.105 (0.597–2.047); 0.751	3.654 (1.515–8.817); 0.004*
Dry mouth	1.514 (1.387–1.653); <0.001*	1.010 (1.009–1.012); <0.001*	0.977 (0.904–1.055); 0.550	1.979 (1.809–2.165); <0.001*
Glossodynia	1.871 (1.575–2.222); <0.001*	1.013 (1.010–1.017); <0.001*	0.913 (0.790–1.056); 0.220	1.632 (1.391–1.914); <0.001*
Swollen tongue	2.566 (2.391–2.753); <0.001*	1.005 (1.004–1.007); <0.001*	0.962 (0.912–1.015); 0.160	1.979 (1.861–2.105); <0.001*
Tongue blistering	2.683 (1.818–3.960); <0.001*	1.014 (1.007–1.020); <0.001*	1.570 (1.186–2.079); 0.002*	2.063 (1.470–2.895); <0.001*
Tongue discomfort	2.929 (2.340–3.665); <0.001*	1.010 (1.006–1.013); <0.001*	0.924 (0.785–1.087); 0.339	7.401 (5.549–9.872); <0.001*
Tongue dry	3.517 (1.922–6.436); <0.001*	1.009 (1.000–1.019); 0.056	0.903 (0.597–1.365); 0.629	2.761 (1.651–4.616); <0.001*
Tongue eruption	2.052 (1.240–3.395); 0.005*	0.998 (0.989–1.007); 0.672	1.221 (0.815–1.830); 0.333	2.677 (1.621–4.421); <0.001*
Tongue erythema	2.194 (1.446–3.328); <0.001*	1.022 (1.013–1.030); <0.001*	0.886 (0.634–1.238); 0.478	4.937 (2.925–8.335); <0.001*
Tongue movement disturbance	1.218 (0.722–2.055); 0.461	0.997 (0.986–1.008); 0.611	0.841 (0.509–1.389); 0.498	2.906 (1.583–5.333); 0.001*
Tongue pruritus	3.272 (2.534–4.226); <0.001*	0.989 (0.985–0.993); <0.001*	0.847 (0.705–1.017); 0.075	7.908 (5.895–10.608); <0.001*
Chapped lips	1.575 (1.208–2.054); 0.001*	1.009 (1.004–1.014); <0.001*	1.046 (0.830–1.319); 0.702	1.712 (1.319–2.221); <0.001*
Lip disorder	1.526 (1.053–2.212); 0.026*	1.004 (0.997–1.012); 0.246	0.786 (0.559–1.105); 0.166	3.032 (1.990–4.617); <0.001*
Lip dry	1.783 (1.322–2.405); <0.001*	1.006 (1.001–1.012); 0.028*	1.035 (0.803–1.333); 0.790	1.972 (1.472–2.641); <0.001*
Lip erythema	1.970 (1.365–2.841); <0.001*	1.002 (0.995–1.008); 0.667	1.060 (0.785–1.433); 0.702	2.861 (1.960–4.176); <0.001*
Lip pain	2.600 (1.954–3.461); <0.001*	1.011 (1.006–1.016); <0.001*	1.057 (0.853–1.309); 0.612	1.842 (1.442–2.353); <0.001*
Lip pruritus	4.514 (3.268–6.235); <0.001*	0.996 (0.992–1.001); 0.125	1.058 (0.868–1.289); 0.578	4.043 (3.086–5.297); <0.001*
Palatal swelling	1.784 (1.101–2.891); 0.019*	1.014 (1.004–1.024); 0.005*	0.864 (0.570–1.309); 0.490	3.249 (1.880–5.615); <0.001*
Aphthous ulcer	1.674 (1.330–2.108); <0.001*	1.004 (0.999–1.008); 0.097	0.998 (0.819–1.216); 0.984	4.003 (3.043–5.265); <0.001*
Circumoral swelling	2.232 (1.081–4.606); 0.030*	0.997 (0.984–1.010); 0.628	1.339 (0.763–2.352); 0.309	3.608 (1.690–7.706); 0.001*
Coating in the mouth	0.702 (0.300–1.643); 0.415	1.034 (1.012–1.057); 0.003*	1.607 (0.697–3.707); 0.266	2.370 (0.793–7.086); 0.122
Mouth swelling	1.920 (1.663–2.216); <0.001*	1.005 (1.002–1.008); <0.001*	1.018 (0.904–1.146); 0.772	3.153 (2.703–3.677); <0.001*
Oral blood blister	0.974 (0.534–1.775); 0.931	1.014 (1.001–1.028); 0.034*	0.867 (0.482–1.562); 0.636	2.885 (1.370–6.077); 0.005*
Oral discomfort	2.921 (2.434–3.506); <0.001*	1.013 (1.010–1.016); <0.001*	1.003 (0.880–1.143); 0.969	2.588 (2.198–3.046); <0.001*
Oral lichen planus	2.708 (1.211–6.055); 0.015*	1.024 (1.009–1.040); 0.001*	0.636 (0.335–1.209); 0.167	3.816 (1.606–9.067); 0.002*
Oral pain	1.453 (1.258–1.678); <0.001*	1.013 (1.010–1.016); <0.001*	0.943 (0.829–1.073); 0.373	1.779 (1.538–2.057); <0.001*
Oral pruritus	3.932 (3.052–5.065); <0.001*	0.997 (0.993–1.000); 0.070	0.921 (0.779–1.089); 0.338	2.486 (2.041–3.029); <0.001*

Data is presented as adjusted odds ratio (95% confidence interval) (*AOR* (95% *CI*)), with the corresponding *p*-value. * statistically significant ($p < 0.05$).

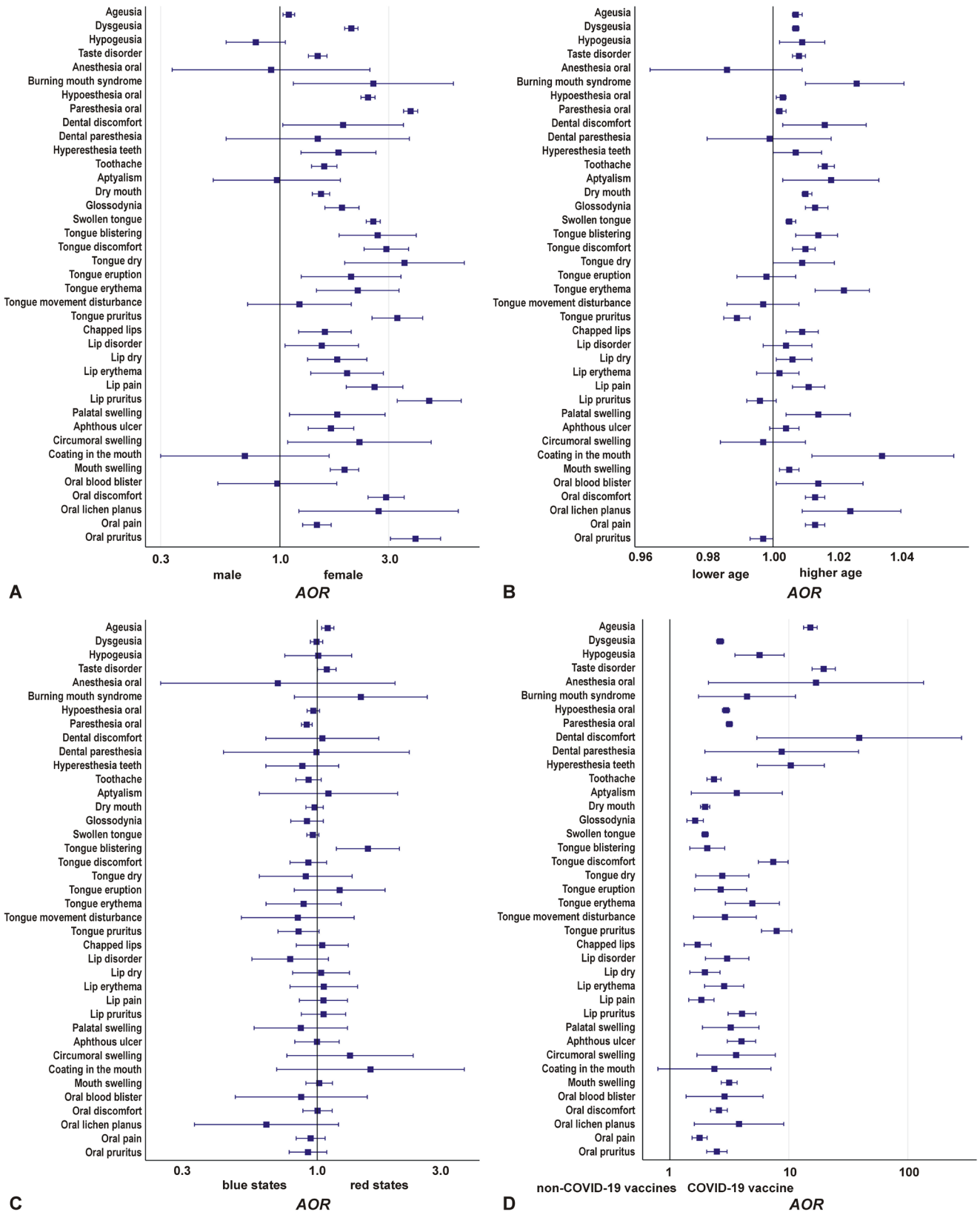

Fig. 4. Logistic regression analysis of oral adverse events (AEs) following coronavirus disease 2019 (COVID-19) vaccination

A. Sex-based disparities; B. Age-based disparities; C. State-level political inclination; D. Vaccine type.
AOR – adjusted odds ratio.

After adding time period to the models, sex-, age- or state-based associations remained stable, while some vaccine-based associations lost statistical significance, including oral anesthesia, burning mouth syndrome and oral lichen planus, among others. The pandemic period was associated with increased odds for 19 SDRs, including taste disorder, ageusia, oral paresthesia, and aphthous ulcer (Table 4).

Table 4. Time-adjusted logistic regression analysis of eligible oral signals of dipropionate reporting (SDRs) associated with coronavirus disease 2019 (COVID-19) vaccination in the United States

Preferred term	Sex (female vs. male)	Age (increase per year)	State (red vs. blue)	Vaccine type (COVID-19 vaccine vs. non-COVID-19 vaccines)	Time period (pandemic vs. pre-pandemic)
Ageusia	1.097 (1.034–1.164); 0.002*	1.007 (1.006–1.009); <0.001*	1.094 (1.035–1.156); 0.001*	9.038 (7.195–11.353); <0.001*	1.978 (1.509–2.592); <0.001*
Dysgeusia	2.056 (1.920–2.201); <0.001*	1.007 (1.006–1.009); <0.001*	0.995 (0.941–1.051); 0.848	3.353 (2.872–3.913); <0.001*	0.750 (0.636–0.884); 0.001*
Hypogeusia	0.778 (0.576–1.051); 0.102	1.009 (1.002–1.016); 0.009*	1.013 (0.751–1.368); 0.931	14.035 (3.412–57.725); <0.001*	0.343 (0.081–1.456); 0.147
Taste disorder	1.478 (1.343–1.626); <0.001*	1.008 (1.006–1.010); <0.001*	1.091 (1.003–1.186); 0.042	4.687 (3.634–6.044); <0.001*	14.917 (8.828–25.206); <0.001*
Anesthesia oral	0.911 (0.336–2.470); 0.855	0.985 (0.962–1.007); 0.184	0.704 (0.248–2.000); 0.510	2.591 (0.335–20.058); 0.362	3,720,842.000 (0.000–Inf); 0.976
Burning mouth syndrome	2.495 (1.110–5.607); 0.027	1.025 (1.009–1.041); 0.002*	1.403 (0.771–2.555); 0.268	1.514 (0.469–4.895); 0.488	5.669 (0.947–33.941); 0.057
Hypoesthesia oral	2.432 (2.264–2.612); <0.001*	1.003 (1.001–1.004); <0.001*	0.966 (0.914–1.021); 0.222	2.690 (2.343–3.089); <0.001*	1.119 (0.963–1.300); 0.144
Paresthesia oral	3.735 (3.477–4.012); <0.001*	1.002 (1.001–1.004); <0.001*	0.910 (0.867–0.955); <0.001*	2.772 (2.459–3.126); <0.001*	1.163 (1.020–1.326); 0.024*
Dental discomfort	2.178 (1.134–4.185); 0.019*	1.013 (1.000–1.026); 0.053	1.136 (0.682–1.893); 0.624	7.388 (1.023–53.343); 0.047*	2,761,506.000 (0.000–Inf); 0.976
Dental paresthesia	1.459 (0.579–3.678); 0.423	0.997 (0.978–1.017); 0.783	0.993 (0.434–2.270); 0.987	1.506 (0.350–6.478); 0.582	6,614,348.000 (0.000–Inf); 0.975
Hyperesthesia teeth	1.785 (1.222–2.607); 0.003*	1.007 (0.999–1.015); 0.079	0.884 (0.639–1.224); 0.458	3.770 (1.554–9.148); 0.003*	4.489 (1.307–15.421); 0.017*
Toothache	1.570 (1.379–1.788); <0.001*	1.016 (1.013–1.019); <0.001*	0.934 (0.833–1.046); 0.238	1.554 (1.238–1.951); <0.001*	1.680 (1.293–2.181); <0.001*
Aptyalism	1.002 (0.517–1.943); 0.994	1.021 (1.005–1.036); 0.008*	1.082 (0.575–2.037); 0.807	18.339 (0.937–358.829); 0.055	0.149 (0.008–2.904); 0.209
Dry mouth	1.501 (1.374–1.639); <0.001*	1.010 (1.009–1.012); <0.001*	0.972 (0.899–1.051); 0.480	2.299 (1.900–2.782); <0.001*	0.827 (0.675–1.014); 0.067
Glossodynia	1.856 (1.562–2.206); <0.001*	1.013 (1.010–1.017); <0.001*	0.917 (0.793–1.062); 0.247	1.661 (1.215–2.272); 0.001*	0.967 (0.691–1.354); 0.846
Swollen tongue	2.572 (2.396–2.761); <0.001*	1.005 (1.004–1.006); <0.001*	0.964 (0.913–1.017); 0.182	1.714 (1.528–1.923); <0.001*	1.194 (1.054–1.353); 0.005*
Tongue blistering	2.737 (1.844–4.064); <0.001*	1.013 (1.006–1.020); <0.001*	1.618 (1.219–2.147); 0.001*	1.196 (0.704–2.030); 0.509	2.026 (1.093–3.755); 0.025*
Tongue discomfort	2.924 (2.333–3.663); <0.001*	1.009 (1.005–1.013); <0.001*	0.918 (0.780–1.082); 0.307	3.371 (2.188–5.193); <0.001*	3.068 (1.763–5.339); <0.001*
Tongue dry	3.423 (1.869–6.270); <0.001*	1.010 (1.000–1.020); 0.043*	0.851 (0.558–1.296); 0.452	2.333 (0.867–6.280); 0.094	1.170 (0.394–3.477); 0.778
Tongue eruption	2.104 (1.256–3.524); 0.005*	0.996 (0.987–1.006); 0.444	1.184 (0.784–1.788); 0.422	1.420 (0.653–3.090); 0.377	2.670 (1.064–6.704); 0.036*
Tongue erythema	2.271 (1.487–3.468); <0.001*	1.021 (1.012–1.029); <0.001*	0.895 (0.640–1.252); 0.518	2.654 (1.180–5.969); 0.018*	2.363 (0.865–6.454); 0.093
Tongue movement disturbance	1.189 (0.704–2.011); 0.517	0.997 (0.986–1.008); 0.586	0.859 (0.519–1.423); 0.555	1.783 (0.646–4.924); 0.264	1.811 (0.578–5.674); 0.308
Tongue pruritus	3.261 (2.525–4.212); <0.001*	0.988 (0.984–0.992); <0.001*	0.841 (0.700–1.011); 0.065	2.486 (1.682–3.673); <0.001*	5.349 (3.138–9.117); <0.001*
Chapped lips	1.630 (1.242–2.139); <0.001*	1.010 (1.005–1.015); <0.001*	1.078 (0.854–1.363); 0.527	2.161 (1.222–3.820); 0.008*	0.767 (0.420–1.400); 0.387
Lip disorder	1.538 (1.056–2.241); 0.025*	1.004 (0.997–1.012); 0.288	0.810 (0.575–1.141); 0.227	3.692 (1.411–9.662); 0.008*	0.763 (0.275–2.117); 0.604
Lip dry	1.829 (1.345–2.485); <0.001*	1.007 (1.001–1.013); 0.018*	1.036 (0.801–1.341); 0.786	1.743 (0.998–3.044); 0.051	1.167 (0.637–2.139); 0.618
Lip erythema	1.940 (1.344–2.799); <0.001*	1.000 (0.993–1.007); 0.979	1.079 (0.798–1.460); 0.620	1.022 (0.624–1.673); 0.932	4.512 (2.361–8.622); <0.001*

Preferred term	Sex (female vs. male)	Age (increase per year)	State (red vs. blue)	Vaccine type (COVID-19 vaccine vs. non-COVID-19 vaccines)	Time period (pandemic vs. pre-pandemic)
Lip pain	2.562 (1.925–3.411); <0.001*	1.010 (1.006–1.015); <0.001*	1.051 (0.847–1.303); 0.651	1.131 (0.767–1.669); 0.535	1.911 (1.223–2.983); 0.004*
Lip pruritus	4.479 (3.242–6.188); <0.001*	0.995 (0.991–1.000); 0.053	1.041 (0.853–1.270); 0.694	1.717 (1.159–2.543); 0.007*	3.264 (2.007–5.307); <0.001*
Palatal swelling	1.781 (1.099–2.887); 0.019*	1.013 (1.003–1.022); 0.011*	0.863 (0.569–1.309); 0.488	1.120 (0.563–2.229); 0.747	5.407 (2.008–14.561); 0.001*
Aphthous ulcer	1.704 (1.349–2.152); <0.001*	1.003 (0.998–1.007); 0.228	0.995 (0.815–1.215); 0.960	2.089 (1.359–3.211); 0.001*	2.480 (1.479–4.157); 0.001*
Circumoral swelling	2.169 (1.049–4.484); 0.037*	0.995 (0.982–1.008); 0.459	1.388 (0.786–2.449); 0.258	0.773 (0.342–1.745); 0.535	17.485 (3.613–84.612); <0.001*
Coating in the mouth	0.702 (0.300–1.644); 0.415	1.033 (1.010–1.056); 0.004*	1.607 (0.697–3.707); 0.266	1.057 (0.246–4.543); 0.940	3.496 (0.492–24.864); 0.211
Mouth swelling	1.915 (1.658–2.212); <0.001*	1.004 (1.001–1.007); 0.006*	1.017 (0.902–1.145); 0.787	1.156 (0.944–1.415); 0.162	4.484 (3.422–5.875); <0.001*
Oral blood blister	0.974 (0.534–1.775); 0.931	1.012 (0.998–1.025); 0.088	0.868 (0.482–1.563); 0.636	0.643 (0.299–1.383); 0.259	33.442 (4.170–268.206); 0.001*
Oral discomfort	2.952 (2.455–3.549); <0.001*	1.013 (1.010–1.016); <0.001*	0.996 (0.873–1.136); 0.950	1.622 (1.241–2.120); <0.001*	1.846 (1.353–2.518); <0.001*
Oral lichen planus	2.633 (1.176–5.896); 0.019*	1.023 (1.007–1.038); 0.004*	0.657 (0.345–1.252); 0.201	2.363 (0.577–9.675); 0.232	2.592 (0.437–15.366); 0.294
Oral pain	1.461 (1.263–1.689); <0.001*	1.013 (1.010–1.016); <0.001*	0.944 (0.828–1.075); 0.383	1.694 (1.282–2.238); <0.001*	1.070 (0.792–1.447); 0.658
Oral pruritus	3.957 (3.066–5.106); <0.001*	0.997 (0.993–1.001); 0.090	0.918 (0.776–1.086); 0.317	3.073 (1.965–4.806); <0.001*	0.765 (0.478–1.224); 0.264

Data is presented as *AOR* (95% *CI*), with the corresponding *p*-value. * statistically significant ($p < 0.05$).

Discussion

Key findings

The disproportionality analysis demonstrated that oral AEs were reported 2.40 times more frequently following COVID-19 vaccination than after all other vaccines in the VAERS database. Females and older adults exhibited higher *PRR*s, whereas severe, hospitalized and fatal cases showed substantially lower *PRR*s. Among the 39 true SDRs, taste-related AEs showed the highest *PRR*s, including taste disorder (23.96), ageusia (17.79), hypogeusia (6.25), and dysgeusia (3.22), alongside other sensory SDRs such as oral paresthesia (3.72) and oral hypoesthesia (3.50).

Sensitivity analyses confirmed that females exhibited a greater number of SDRs (32/39) than males (21/39). Middle-aged adults showed the highest number of true SDRs, whereas children (<5 years) and the elderly (≥80 years) exhibited the fewest. Non-severe (30/39), non-hospitalized (34/39) and non-fatal (36/39) cases accounted for substantially more SDRs than severe (8/39), hospitalized (2/39) and fatal cases (0/39). The mRNA-based (35/39) and viral vector-based (25/39) vaccines were associated with more SDRs than protein subunit COVID-19 vaccines (6/39) and influenza vaccines (0/39). Co-occurrence analyses demonstrated moderate agreement between gustatory and olfactory AEs ($\kappa = 0.459$), which increased for non-COVID-19 vaccines during the pandemic ($\kappa = 0.500$) compared with the pre-pandemic period ($\kappa = 0.115$). This pattern suggests the presence of notoriety bias rather than a causal association with COVID-19 vaccines.

Regression analyses confirmed that female sex was associated with significantly higher odds for 32 true SDRs, and that increasing age was associated with a higher probability of developing 27 SDRs. State-level political inclination had no significant influence on SDR reporting, except for 3 SDRs. The inclusion of time period as a predictor did not alter sex-, age- or state-based associations, indicating that notoriety bias did not substantially affect these patterns. However, adjustment for time attenuated the associations for several SDRs, particularly burning mouth syndrome, oral anesthesia and oral lichen planus, suggesting that reporting trends influenced the strength of association for certain AEs over time.

Sex- and age-related disparities

The higher susceptibility of females to reporting oral AEs following COVID-19 vaccination aligns with previous pharmacovigilance studies involving other organ systems.[24] Disproportionality analyses using the WHO VigiBase database have consistently shown that females are at least twice as likely to report autoimmune,[25] neurologic[26,27] and cutaneous AEs[28,29] following COVID-19 vaccination.[25–29] This sex disparity may be attributed to

both biological and behavioral factors.[30] Sex hormones, particularly estrogen, enhance immune responses and contribute to greater reactogenicity, while chromosomal differences further modulate immune function.[31] Additionally, higher baseline prevalence of certain conditions in females, coupled with increased health-seeking and reporting behaviors, may amplify these disparities.[30]

The observed age-related patterns are consistent with prior analyses indicating that adults, especially middle-aged individuals, are more susceptible to AEs following COVID-19 vaccination than minors (≤18 years) and seniors (≥64 years).[25,32,33] Increase in AE reporting with age has been documented for conditions such as herpes zoster, myocardial infarction and circulatory collapse.[28,34] In contrast, some studies have identified a reversed pattern, where certain AEs, including myocarditis/pericarditis, thrombosis/thrombocytopenia, and anaphylaxis were more frequently reported in younger populations, while others found no significant age-related differences.[35–40]

State-level political inclination

Political preferences have been shown to influence perceptions of vaccine safety.[3,41–43] A recent US cross-sectional study reported that while vaccinated individuals across political groups experienced similar AEs, Republicans were more likely to perceive these events as severe, report higher rates of negative reactions among their social circles, and discourage others from vaccination.[41] The study highlights the role of political ideology in shaping vaccine perceptions even among the vaccinated.[41] Consistently, Asch et al. performed a disproportionality analysis of COVID-19 vaccine AEs in VAERS, revealing that a 10% increase in Republican voting was associated with a 5% rise in overall AE reporting, a 25% increase in severe AE reports, and a 21% higher likelihood that any reported AE was classified as severe.[42] Moreover, partisan divisions over COVID-19 vaccines are speculated to erode public support for other vaccination programs, including childhood immunization mandates.[43] Media politicization may further reinforce these trends, as right-leaning outlets were more likely to emphasize the risks of COVID-19 vaccine-associated myocarditis (CVM) without contextualizing its rarity or comparing it to the risks of myocarditis from SARS-CoV-2 infection, whereas left-leaning media were highlighting the benefits of vaccination and provided numerical risk estimates to contextualize CVM concerns.[3] In the present analysis, state-level political inclination was used as a proxy for political orientation, as previously described.[42] No strong association was noted between political affiliation and oral AE reporting patterns.[42]

Vaccine schedule and type

Primary vaccination doses were associated with higher AE reporting rates than booster doses, which is consistent with previous evidence of greater reactogenicity among dose-naïve individuals.[17,44] The mRNA vaccines exhibited more SDRs than vector-based or protein subunit vaccines, which aligns with previous reports highlighting greater reactogenicity of mRNA platforms.[45,46]

Are taste-related AEs a manifestation of notoriety bias?

The strong association between chemosensory disorders, particularly gustatory and olfactory dysfunction, and the SARS-CoV-2 infection may have increased public vigilance of these symptoms due to their diagnostic and prognostic relevance.[13–15] As a result, sporadic cases of gustatory and olfactory dysfunction following COVID-19 vaccination have been reported.[16,47] In response to this heightened suspicion, active surveillance studies funded by the European Medicines Agency (EMA), including SAFETY–VAC, COVID-19 Vaccine Monitor (CVM), and Early COVID-19 Vaccine Monitor (ECVM), included anosmia and ageusia among their AEs of interest.[48,49] Surprisingly, evidence from these cohort studies indicated that the observed rates of anosmia and ageusia decreased following COVID-19 vaccination compared to the background rates, which had been severely elevated during the COVID-19 pandemic.[50–52]

In passive surveillance databases, chemosensory AEs were increasingly reported following COVID-19 vaccination, raising alternative hypotheses beyond a direct causal link to the vaccine, including coincidental or breakthrough infection.[7–9] Both scenarios may be influenced by notoriety bias. Our findings partially support the notoriety bias hypotheses, as we observed a weak co-occurrence of gustatory and olfactory AEs before COVID-19, which became more pronounced during the pandemic for both COVID-19 and non-COVID-19 vaccines. Also, the crude reporting rate of taste-related AEs increased several-fold during the pandemic.[7] Furthermore, the strengthened correlation between Google search activity and VAERS reporting after vaccine rollout was observed.

Strengths

The present study has several strengths. The hybrid analytical approach employed is strongly recommended, as no single data-mining method is inherently superior; therefore, combining multiple approaches is essential for identifying the most reliable SDRs.[4,53] Additionally, comprehensive stratification and sensitivity analyses across sex, age, clinical course, political inclination, vaccine type, and time period offer deeper insights into oral AE patterns and help identify potential reporting biases.[4] The suspected notoriety bias associated with taste-related AEs was investigated using an innovative framework that incorporated stratified co-occurrence analysis and real-time digital search data. Moreover, this study is among

the few to examine the role of political determinants in AE reporting.[42]

Limitations

This study has several limitations. First, reliance on passive surveillance data from VAERS is subject to inherent biases, including underreporting, overreporting and the inability to establish causal relationships between vaccines and reported AEs.[12] As with all disproportionality analyses, causality cannot be inferred from statistical associations alone. Second, while the hybrid frequentist–Bayesian approach enhances the robustness of signal detection, disproportionality analysis alone cannot determine the clinical relevance or biological mechanisms underlying observed SDRs.[5] Third, state-level political inclination was used as a proxy for individual political preferences, which may not fully capture the complexity of political influences on AE reporting.[42] Finally, Google Trends data was utilized as an external indicator of notoriety bias. However, extensive media coverage and heightened public awareness, especially during the pandemic, may have contributed to the overestimation of certain SDRs, especially taste-related AEs.

Conclusions

The findings of the present study underscore the need for granular evaluation of clinically relevant oral SDRs, such as oral lichen planus, using self-controlled case-series methodologies to validate them as true safety signals.[54] Given the potential role of notoriety bias in chemosensory AE reporting, future disproportionality analyses should incorporate sensitivity analyses and innovative analytical approaches to minimize its impact. Furthermore, as most oral AEs were strongly associated with favorable clinical outcomes (non-severe, non-hospitalized and non-fatal), an important public health message emerges: although oral AEs following COVID-19 vaccination may occur, they should not be perceived as life-threatening or abused by antivaccine propaganda to undermine public confidence in vaccine safety.

Ethics approval and consent to participate

Not applicable.

Data availability

Publicly available datasets were analyzed in this study. This data can be found at https://vaers.hhs.gov/data/datasets.html. The datasets supporting the findings of the current study are openly available in the Open Science Framework (OSF) at https://doi.org/10.17605/OSF.IO/3S8KW.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Generative AI was used in the preparation of the manuscript. Specifically, ChatGPT (v. 4; OpenAI, San Francisco, USA) was employed for proofreading and language refinement. This tool was used solely to improve grammar and readability. All substantive content was independently developed by the authors.

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References

1. Siani A. Vaccine Hesitancy and Refusal: History, Causes, Mitigation Strategies. In: Rezaei N, ed. *Integrated Science of Global Epidemics*. Cham, Switzerland: Springer International Publishing; 2023:503–517. doi:10.1007/978-3-031-17778-1_23
2. Riad A. *COVID – 19 Vaccine Hesitancy: A Tale of Two Pandemics* [dissertation]. Prague, Czech Republic: Charles University; 2024. https://dspace.cuni.cz/handle/20.500.11956/189034. Accessed May 22, 2024.
3. Matsumura A, Garg R, Hussain M, Matsumura ME. Political orientation of online media sources and reporting of Covid-19 vaccine myocarditis. *PLoS One*. 2024;19(1):e0296295. doi:10.1371/JOURNAL.PONE.0296295
4. Cutroneo PM, Sartori D, Tuccori M, et al. Conducting and interpreting disproportionality analyses derived from spontaneous reporting systems. *Front Drug Saf Regul*. 2024;3:1323057. doi:10.3389/FDSFR.2023.1323057
5. European Medicines Agency. Guideline on good pharmacovigilance practices (GVP) – Module IX Addendum I – Methodological aspects of signal detection from spontaneous reports of suspected adverse reactions. 2017. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-pharmacovigilance-practices-gvp-module-ix-addendum-i-methodological-aspects-signal-detection-spontaneous-reports-suspected-adverse-reactions_en.pdf. Accessed February 14, 2025.
6. Vaccine Adverse Event Reporting System (VAERS). Guide to Interpreting VAERS Data. https://vaers.hhs.gov/data/dataguide.html. Accessed January 28, 2025.
7. Riad A, Põld A, Kateeb E, Attia S. Oral adverse events following COVID-19 vaccination: Analysis of VAERS reports. *Front Public Health*. 2022;10:952781. doi:10.3389/FPUBH.2022.952781
8. Riad A, Schulz-Weidner N, Dziedzic A, Howaldt HP, Attia S. Oral side effects of COVID-19 vaccines in 32 European countries: Analysis of EudraVigilance reports. *J Med Virol*. 2023;95(5):e28771. doi:10.1002/JMV.28771
9. Riad A, Issa J, Attia S, Dušek L, Klugar M. Oral adverse events following COVID-19 and influenza vaccination in Australia. *Hum Vaccin Immunother*. 2023;19(2):2253589. doi:10.1080/21645515.2023.2253589
10. Abubakr N, Salem ZA, Kamel AHM. Oral manifestations in mild-to-moderate cases of COVID-19 viral infection in the adult population. *Dent Med Probl*. 2021;58(1):7–15. doi:10.17219/DMP/130814
11. Mazur M, Duś-Ilnicka I, Jedliński M, et al. Facial and oral manifestations following COVID-19 vaccination: A survey-based study and a first perspective. *Int J Environ Res Public Health*. 2021;18(9):4965. doi:10.3390/IJERPH18094965
12. Faillie JL. Case–non-case studies: Principle, methods, bias and interpretation. *Therapies*. 2019;74(2):225–232. doi:10.1016/J.THERAP.2019.01.006
13. Agyeman AA, Chin KL, Landersdorfer CB, Liew D, Ofori-Asenso R. Smell and taste dysfunction in patients with COVID-19: A systematic review and meta-analysis. *Mayo Clin Proc*. 2020;95(8):1621–1631. doi:10.1016/J.MAYOCP.2020.05.030

14. Menni C, Valdes AM, Freidin MB, et al. Real-time tracking of self-reported symptoms to predict potential COVID-19. *Nat Med*. 2020;26(7):1037–1040. doi:10.1038/s41591-020-0916-2
15. Lee Y, Min P, Lee S, Kim SW. Prevalence and duration of acute loss of smell or taste in COVID-19 patients. *J Korean Med Sci*. 2020;35(18):e174. doi:10.3346/JKMS.2020.35.E174
16. Tsuchiya H, Mizogami M. Characteristics of oral adverse effects following COVID-19 vaccination and similarities with oral symptoms in COVID-19 patients: Taste and saliva secretory disorders. *Med Princ Pract*. 2025;34(2):101–120. doi:10.1159/000543182
17. Moballegh Naseri M, Moballegh Naseri M, Maurya VK, Shams S, Pitaloka DAE. Epitope-based vaccine design against the membrane and nucleocapsid proteins of SARS-CoV-2. *Dent Med Probl*. 2023;60(3):489–495. doi:10.17219/DMP/161742
18. Fusaroli M, Salvo F, Begaud B, et al. The REporting of A Disproportionality Analysis for DrUg Safety Signal Detection Using Individual Case Safety Reports in PharmacoVigilance (READUS-PV): Explanation and elaboration. *Drug Saf*. 2024;47(6):585–599. doi:10.1007/S40264-024-01423-7
19. Vaccine Adverse Event Reporting System (VAERS). About VAERS. https://vaers.hhs.gov/about.html. Accessed January 27, 2025.
20. MedDRA. Medical Dictionary for Regulatory Activities. MedDRA Hierarchy. https://www.meddra.org/how-to-use/basics/hierarchy. Accessed January 27, 2025.
21. United States® Census Bureau. Exploring Age Groups in the 2020 Census. https://www.census.gov/library/visualizations/interactive/exploring-age-groups-in-the-2020-census.html. Accessed January 28, 2025.
22. National Archives. 2020 Electoral College Results. https://www.archives.gov/electoral-college/2020. Accessed January 28, 2025.
23. The R Foundation. The R Project for Statistical Computing. https://www.r-project.org/index.html. Accessed May 22, 2023.
24. Sachdeva S, Saluja H, Mani A. Cognizance, adverse effects and motivation regarding COVID-19 vaccination amongst health care professionals: A cross-sectional study. *Dent Med Probl*. 2022;59(1):13–19. doi:10.17219/DMP/145757
25. Kim S, Bea S, Choe SA, Choi NK, Shin JY. Autoimmune disorders reported following COVID-19 vaccination: A disproportionality analysis using the WHO database. *Eur J Clin Pharmacol*. 2024;80(3):445–453. doi:10.1007/S00228-023-03618-W
26. Dutta S, Kaur R, Charan J, et al. Analysis of neurological adverse events reported in VigiBase from COVID-19 vaccines. *Cureus*. 2022;14(1):e21376. doi:10.7759/CUREUS.21376
27. Kim JE, Park J, Song TJ. A disproportionality analysis for the association of central nervous system demyelinating diseases with COVID-19 vaccination using the World Health Organization pharmacovigilance database. *Mult Scler*. 2022;28(13):2112–2123. doi:10.1177/13524585221109397
28. Préta LH, Contejean A, Salvo F, Treluyer JM, Charlier C, Chouchana L. Association study between herpes zoster reporting and mRNA COVID-19 vaccines (BNT162b2 and mRNA-1273). *Br J Clin Pharmacol*. 2022;88(7):3529–3534. doi:10.1111/BCP.15280
29. The HL, Keowmani T, Tang MM. Risk factors associated with cutaneous reactions following COVID-19 vaccine immunisation: A registry-based case–control study. *Malay J Med Sci*. 2024;31(3):133–148. doi:10.21315/mjms2024.31.3.10
30. Duijster JW, Lieber T, Pacelli S, et al. Sex-disaggregated outcomes of adverse events after COVID-19 vaccination: A Dutch cohort study and review of the literature. *Front Immunol*. 2023;14:1078736. doi:10.3389/FIMMU.2023.1078736
31. Flanagan KL, Fink AL, Plebanski M, Klein SL. Sex and gender differences in the outcomes of vaccination over the life course. 2017;33:577–599. doi:10.1146/ANNUREV-CELLBIO-100616-060718
32. Kim JE, Park J, Min YG, Hong YH, Song TJ. Associations of neuralgic amyotrophy with COVID-19 vaccination: Disproportionality analysis using the World Health Organization pharmacovigilance database. *Muscle Nerve*. 2022;66(6):766–770. doi:10.1002/MUS.27734
33. Chen C, Fu F, Ding L, Xiao J. Hearing disorder following COVID-19 vaccination: A pharmacovigilance analysis using the Vaccine Adverse Event Reporting System. *J Clin Pharm Ther*. 2022;47(11):1789–1795. doi:10.1111/JCPT.13767
34. Kaur RJ, Dutta S, Charan J, et al. Cardiovascular adverse events reported from COVID-19 vaccines: A study based on WHO database. *Int J Gen Med*. 2021;14:3909–3927. doi:10.2147/IJGM.S324349
35. Foltran D, Delmas C, Flumian C, et al. Myocarditis and pericarditis in adolescents after first and second doses of mRNA COVID-19 vaccines. *Eur Heart J Qual Care Clin Outcomes*. 2022;8(2):99–103. doi:10.1093/EHJQCCO/QCAB090
36. Li M, Yuan J, Lv G, Brown J, Jiang X, Lu ZK. Myocarditis and pericarditis following COVID-19 vaccination: Inequalities in age and vaccine types. *J Pers Med*. 2021;11(11):1106. doi:10.3390/JPM11111106
37. Polack FP, Thomas SJ, Kitchin N, et al.; C4591001 Clinical Trial Group. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Eng J Med*. 2020;383(27):2603–2615. doi:10.1056/NEJMOA2034577
38. Oster ME, Shay DK, Su JR, et al. Myocarditis cases reported after mRNA-based COVID-19 vaccination in the US from December 2020 to August 2021. *JAMA*. 2022;327(4):331–340. doi:10.1001/JAMA.2021.24110
39. Shimabukuro T, Nair N. Allergic reactions including anaphylaxis after receipt of the first dose of Pfizer-BioNTech COVID-19 vaccine. *JAMA*. 2021;325(8):780–781. doi:10.1001/JAMA.2021.0600
40. Schultz NH, Sørvoll IH, Michelsen AE, et al. Thrombosis and thrombocytopenia after ChAdOx1 nCoV-19 vaccination. *New Eng J Med*. 2021;384(22):2124–2130. doi:10.1056/NEJMOA2104882
41. Farabee D, Hawken A. Perceptions of COVID-19 vaccine side effects by political affiliation. *J Public Health (Oxf)*. 2023;45(4):930–934. doi:10.1093/pubmed/fdad105
42. Asch DA, Luo C, Chen Y. Reports of COVID-19 vaccine adverse events in predominantly Republican vs Democratic states. *JAMA Netw Open*. 2024;7(3):e244177. doi:10.1001/jamanetworkopen.2024.4177
43. Motta M. Is partisan conflict over COVID-19 vaccination eroding support for childhood vaccine mandates? *NPJ Vaccines*. 2023;8(1):5. doi:10.1038/s41541-023-00611-3
44. Shin J, Shim SR, Lee J, Ryu HS, Kim JY. Otorhinolaryngologic complications after COVID-19 vaccination, Vaccine Adverse Event Reporting System (VAERS). *Front Public Health*. 2024;11:1338862. doi:10.3389/FPUBH.2023.1338862
45. di Mauro G, Mascolo A, Longo M, et al. European safety analysis of mRNA and viral vector COVID-19 vaccines on glucose metabolism events. *Pharmaceuticals*. 2022;15(6):677. doi:10.3390/PH15060677
46. Ruggiero R, Donniacuo M, Mascolo A, et al. COVID-19 vaccines and atrial fibrillation: Analysis of the post-marketing pharmacovigilance European database. *Biomedicines*. 2023;11(6):1584. doi:10.3390/BIOMEDICINES11061584
47. Arabzadeh Bahri R, Esmaeilpur Abianeh F, Peisepar M, et al. Anosmia or ageusia following COVID-19 vaccination: A systematic review. *Ear Nose Throat J*. 2024;103(1_suppl):164S–170S. doi:10.1177/01455613241233098
48. VAC4EU. EMA-funded studies. Projects and Studies. https://vac4eu.org/projects-and-studies. Accessed February 14, 2025.
49. Egbers T, Willame C, Belbachir L, et al. ACCESS-Background rate of adverse events-definition – anosmia & ageusia. doi:10.5281/ZENODO.5236687
50. Sturkenboom MCJM, Messina D, Paoletti O, et al. Cohort monitoring of adverse events of special interest and COVID-19 diagnoses prior to and after COVID-19 vaccination. doi:10.5281/ZENODO.6762311
51. Durán CE, Messina D, Gini R, et al. Rapid safety assessment of SARS-CoV-2 vaccines in EU member states using electronic health care data sources (COVID vaccine monitor-CVM study): Final study report for WP3 (electronic health record data). doi:10.5281/ZENODO.10093613
52. Gallagher TJ, Parikh M, Herrera K, Lin ME, Hur K. Association of COVID-19 vaccination with changes in smell and taste. *Otolaryngol Head Neck Surg*. 2024;171(4):1240–1244. doi:10.1002/OHN.833
53. Park G, Jung H, Heo SJ, Jung I. Comparison of data mining methods for the signal detection of adverse drug events with a hierarchical structure in postmarketing surveillance. *Life (Basel)*. 2020;10(8):138. doi:10.3390/LIFE10080138
54. Whitaker HJ, Hocine MN, Farrington CP. The methodology of self-controlled case series studies. *Stat Methods Med Res*. 2009;18(1):7–26. doi:10.1177/0962280208092342

Original paper

Implications of orthodontic treatment on esthetics, oral health and quality of life in adult patients

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Abstract

Background. The coronavirus disease 2019 (COVID-19) pandemic has had a profound impact on people's daily lives, resulting in lifestyle changes, social distancing, isolation, as well as economic consequences. An evaluation of the effects of COVID-19 on oral health is necessary.

Objectives. The aim of the study was to evaluate the effects of fixed orthodontic treatment on periodontal health and quality of life in adult patients.

Material and methods. Due to the COVID-19 pandemic, the study methodology was based on a questionnaire consisting of 20 items addressed to patients undergoing orthodontic treatment. The questions concerned the frequency and duration of toothbrushing, brushing techniques as well as the use of auxiliary means of oral hygiene, with the objective of drawing conclusions regarding the oral health of this patient population. Additionally, 5 items adapted from the Oral Health Impact Profile-5 (OHIP-5) were included, asking subjects how frequently they had experienced specific problems during orthodontic treatment.

Results. The oral health of adult patients undergoing orthodontic treatment was influenced by the technique, frequency and means of toothbrushing. During fixed orthodontic treatment, pathological changes in periodontal tissues were often observed (23.6–56.4%), along with other conditions influencing patients' quality of life (10.2–81.3%).

Conclusions. Early diagnosis of periodontal conditions during orthodontic treatment is essential for treatment success. Orthodontists should instruct patients on appropriate oral hygiene practices and on detecting changes in periodontal status, as well as should recommend periodontal consultation when needed. Orthodontic treatment in adult patients, through esthetic and functional improvement, should lead to an improvement in psychosocial wellbeing and an increase in quality of life.

Keywords: quality of life, oral health, esthetics, orthodontic treatment, periodontal structures

Highlights

- Orthodontic treatment in adults is associated with measurable changes in periodontal and alveolar bone parameters, with gingival alterations frequently observed during therapy.
- Periodontal outcomes during orthodontic treatment are more strongly associated with effective oral hygiene technique than with brushing frequency alone; adjunctive aids such as interdental brushes play a critical role in maintaining oral hygiene.
- The COVID-19 pandemic was associated with a reduced quality of life among orthodontic patients, contributing to both functional limitations and psychosocial distress during treatment.
- Early identification and management of periodontal complications are essential for optimal treatment outcomes, and continuous patient education is a key factor in achieving long-term therapeutic success.

Introduction

Orthodontic treatment has increasingly been applied to improve esthetic appearance in adult patients. Orthodontic treatment is used to stabilize occlusion and modify tooth position, if necessary. These procedures must be performed professionally, as inadequate treatment may lead to functional impairments, including difficulties in speech.[1,2] The aim of orthodontic treatment is to obtain esthetically acceptable results, functional occlusion and appropriate tooth movement, i.e., the interaction between the teeth and the periodontal tissues.[3,4] Previous studies have demonstrated that educating parents in oral and periodontal hygiene can lead to less dental and periodontal complications during orthodontic treatment.[1–5] However, regular professional consultations are essential to prevent complex orthodontic–periodontal conditions.[1–5] Dental braces are commonly used to correct tooth misalignment,[4,5] and various prophylactic measures have been introduced to improve the quality of orthodontic treatment.[6–8]

Early detection of periodontal conditions is essential for successful orthodontic therapy. Therefore, comprehensive periodontal examinations should be performed before, during and after treatment, including the assessment of the plaque index (PI), papillary bleeding index (PBI), periodontal pocket depth (PD), and radiographic evaluation.[2,9,10]

Patients should be instructed to maintain proper oral hygiene and effective plaque control, and clinicians should carefully monitor their patients' health.[7–11] In adult patients, radiological examination aimed at detecting carious lesions and bone or root resorption should be performed at least once a year. The benefits of brushing teeth twice a day and using an ultrasonic toothbrush[12] or an oscillating-rotating power toothbrush[13] over a manual toothbrush have been demonstrated, particularly when combined with appropriate brushing techniques[14] and regular interdental cleaning.[11,15] To protect gingival tissues and reduce bacterial load, chlorhexidine may be administered in gel[16] or mouthwash form.[17]

If clinical or radiologic examinations reveal pathological changes in superficial or deep periodontal structures, patients should be referred to a periodontology specialist for a consultation. Orthodontic treatment should be initiated only in the presence of an intact or reduced periodontium, as treatment in the presence of pre-existing periodontal lesions may exacerbate existing lesions and compromise outcomes.

Before initiating orthodontic treatment, clinicians must ensure that patients are capable of maintaining consistent and efficient oral hygiene practices. If adequate plaque control cannot be achieved, treatment should be postponed until satisfactory oral hygiene is established. Patient awareness regarding oral health maintenance is essential for achieving optimal treatment results.

The aim of the present study was to evaluate the effects of fixed orthodontic treatment on periodontal health and quality of life in adult patients, to assess potential changes in periodontal structures, and to examine how orthodontists address these issues in clinical practice.

Material and methods

Due to the restrictions associated with the coronavirus disease 2019 (COVID-19) pandemic, this study was conducted using a questionnaire. The questionnaire consisted of 20 items and was designed to be easily understood by participants. The target population comprised adult patients who were currently undergoing or had previously undergone fixed orthodontic treatment with brackets on one or both dental arches. The first part of the questionnaire included questions about the frequency and duration of toothbrushing, brushing techniques, and the use of auxiliary oral hygiene aids. Given the lack of clinical examinations, additional questions were included to allow indirect assessment of periodontal health. To improve comprehensibility, colloquial expressions were used where appropriate (e.g., "growth in volume of the gums" for gingival edema of bacterial cause or "bad breath" for halitosis). Additionally, 5 items adapted from the Oral

Health Impact Profile-5 (OHIP-5) were incorporated. Participants indicated the frequency of experiencing each problem (functional limitation, physical pain, psychological discomfort, physical disability, social disability) during orthodontic treatment using a five-point Likert scale, as follows: 0 – never; 1 – hardly ever; 2 –occasionally; 3 – fairly often; and 4 – very often. In addition to questions evaluating the effects of orthodontic treatment, the questionnaire included items assessing the clinical measures undertaken by orthodontists in response to observed changes in periodontal structures.

A total of 165 patients of both sexes, aged between 19 and 58 years, from Târgu Mureș and Sibiu, Romania, completed the questionnaire. Based on the previous studies,[2,9] the examination protocol presented in Table 1 was adopted.

Results

Of the 165 respondents, 139 (84.2%) were female and 26 (15.8%) were male. Ninety-one participants (55.2%) had completed their orthodontic treatment and reported the duration of therapy.

Regarding oral hygiene practices, 108 patients (65.5%) used a manual toothbrush, 48 (29.1%) used an orthodontic toothbrush and 51 (30.9%) used an electric toothbrush. Most participants (126 (76.4%)) reported brushing their teeth twice a day, 33 (20.0%) brushed after every meal and 6 (3.6%) brushed once daily. In terms of brushing techniques, 6 patients (3.6%) used horizontal movements, 33 (20.0%) brushed vertically, 57 (34.5%) used circular movements, and 69 (41.8%) applied a combined technique. With respect to auxiliary means of removing oral biofilm, 105 individuals (63.6%) used dental floss, 102 (61.8%) – mouthwash, 102 (61.8%) – interdental brush, 36 (21.8%) – oral irrigator, and 21 (12.7%) – toothpicks (Fig. 1).

Orthodontic treatment was reported to make oral hygiene maintenance difficult by 96 patients (58.2%). However, 126 participants (76.4%) stated that they improved their oral hygiene techniques and plaque control measures during orthodontic treatment.

Table 1. Periodontal examination parameters evaluated before, during and after orthodontic treatment

Time point	PI	PBI	PD	Rx
Before orthodontic treatment	+	+	+	+
During orthodontic treatment	+ 1/month	+ 1/6 months	+ 1/6 months	+ 1/12 months
After orthodontic treatment	+ 1/6 months	+ 1/12 months	+ 1/12 months	+ when PBI and PD change

PI – plaque index; PBI – papillary bleeding index; PD – periodontal pocket depth; Rx – radiographic examination.

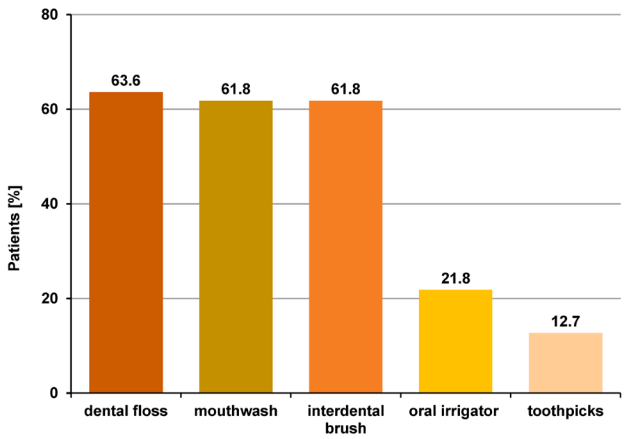

Fig. 1. Use of auxiliary oral hygiene aids among study participants

Over the course of treatment, 90 patients (54.5%) observed changes in the color of their gingiva to red, 85 (51.5%) reported increased gingival volume, 39 (23.6%) experienced gingival retraction, and 93 (56.4%) participants reported spontaneous bleeding or bleeding during brushing (Fig. 2).

Sixty-six (40.0%) patients noticed the accumulation of calculus during treatment and underwent scaling. In response to the question "Have you had difficulty chewing certain foods because of orthodontic treatment?", participants answered as follows: never (1 (0.6%)); hardly ever (4 (2.4%)); occasionally (15 (9.1%)); fairly often (134 (81.2%)); and very often (11 (6.7%)). Pain, secondary to orthodontic treatment, was reported as follows: hardly ever (8 (4.8%)); occasionally (21 (12.7%)); fairly often (94 (57.0%)); and very often (42 (25.5%)) (Fig. 3).

For the question "Have you felt that food had less flavor because of fixed orthodontic treatment?", the responses were: never (23 (13.9%)); hardly ever (42 (25.5%)); occasionally (67 (40.6%)); fairly often (22 (13.3%)); and very often (11 (6.7%)).

When asked whether they felt uncomfortable about the appearance of their teeth, mouth or dentures, participants

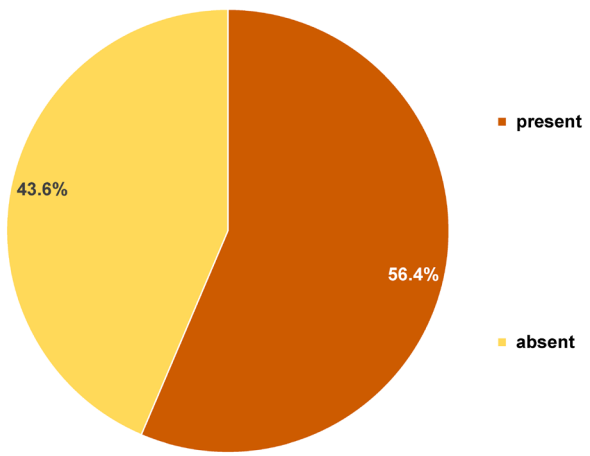

Fig. 2. Self-reported gingival bleeding among respondents during orthodontic treatment

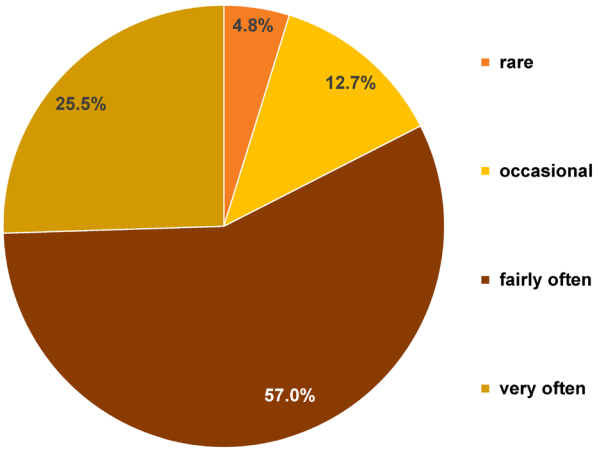

Fig. 3. Self-reported pain associated with orthodontic treatment among respondents

responded as follows: never (51 (30.9%)); hardly ever (34 (20.6%)); occasionally (38 (23.0%)); fairly often (17 (10.3%)); and very often (25 (15.2%)). Regarding difficulty performing usual daily activities because of orthodontic treatment, the answers were: never (18 (10.9%)); hardly ever (47 (28.5%)); occasionally (53 (32.1%)); fairly often (38 (23.0%)); and very often (9 (5.5%)).

Among patients who reported anatomical or functional changes in periodontal structures during orthodontic treatment, 48 (29.1%) stated that these changes were noted or managed by their orthodontist. In addition, 9 patients (5.5%) reported being referred to or treated by a periodontology specialist before or during orthodontic treatment, as recommended by their orthodontist.

Discussion

By analyzing the responses to the questionnaire, we were able to assess oral health status of adult patients undergoing orthodontic treatment. The occurrence of pathological changes in the gingiva during orthodontic therapy is closely associated with the quality of oral hygiene practices. Therefore, the questionnaire included several items addressing brushing techniques and auxiliary oral hygiene measures.

In the present study, the majority of participants used a manual toothbrush (65.5%), while 30.9% used an electric toothbrush. Gingival color changes were reported by 54.5% of patients, gingival volume growth by 51.5%, gingival retractions by 23.6%, and spontaneous bleeding or bleeding during toothbrushing by 56.4%. These gingival alterations were more frequently reported by individuals who used electric toothbrushes.

The efficiency of manual toothbrushing compared to electric toothbrushing was assessed by evaluating the PI and gingival inflammation before and after 30 days of brushing.[6] The authors concluded that manual toothbrushes were more effective than ultrasonic toothbrushes in removing plaque around orthodontic appliances, although no significant difference in gingival status were observed. Other studies have reported that electric toothbrushing may be superior, although differences in plaque removal are not significant.[18–20]

Most participants in the present study reported brushing their teeth after major meals (33 (20.0%)), two times a day (126 (76.4%)) or once a day (6 (3.6%)), indicating a generally high level of oral hygiene awareness. A similar study performed by Pandey et al. showed that 63% of patients undergoing orthodontic treatment brush their teeth once per day, 26% brush twice a day, and 11% – three times a day.[7]

Our results also showed that patients who used horizontal or combined brushing techniques experience gingival inflammation more frequently that those using vertical movements. Given the lack of consensus regarding optimal brushing techniques, Nassar et al. compared horizontal, Stillman and Bass methods in 30 adult patients over 9 months and found that the Bass technique was most effective in reducing PI and gingival inflammation in patients with fixed orthodontic appliances.[9]

Of the patients who responded to our questionnaire, 105 (63.6%) used dental floss, 102 (61.8%) – mouthwash, 102 (61.8%) – interdental brush, 36 (21.8%) – oral irrigator, and 21 (12.7%) – toothpicks. Individuals who used auxiliary oral hygiene aids reported fewer inflammatory gingival changes, suggesting improved plaque control. Previous studies have shown that combining manual and interdental brushing provides better plaque control and reduces gingival inflammation compared with manual brushing alone.[15]

Bock et al. compared cylindrical and cylindro-conical interdental brushes and found significant reductions in plaque levels with both types, but no significant difference was observed between them.[12]

Patients undergoing fixed orthodontic treatment are particularly susceptible to gingival inflammation due to difficulties in mechanical plaque removal. Therefore, these individuals would benefit from the use of mouthwashes. Chlorhexidine-based products are widely used to reduce oral bacterial load and prevent or treat some forms of gingivitis and periodontitis.[16]

Mazzoleni et al. observed that the water jet of the oral irrigator does not significantly improve the efficiency of oral hygiene in patients with orthodontic treatment. However, these patients did not present the traditional aggravation of periodontal status throughout the entire orthodontic treatment.[17] Oral irrigators represent useful adjuncts for reducing gingival inflammation despite their limited effects on plaque accumulation.[21] In our study, most participants (76.4%) reported an enhancement in their oral hygiene practices during orthodontic treatment. Petrauskiene et al. noted significant modifications in oral hygiene habits among patients with braces, while the majority of patients wearing removable orthodontic apparatus did not change their habits.[2]

Orthodontic treatment may improve periodontal status, but adverse effects during therapy are relatively frequent. In our sample, 85 patients observed the increase in gingival volume and 39 participants reported gingival retraction. Zhao et al. noted increased gingival volume or gingival retraction in patients undergoing orthodontic treatment,[10] while Verrusio et al. reported increased periodontal parameters following orthodontic treatment, which is likely related to changes in subgingival microbial flora.[22] These modifications can subsequently induce inflammation and papillary bleeding.[22]

Plaque accumulation remains a major secondary effect of fixed orthodontic treatment due to long-term difficulties in maintaining adequate oral hygiene. In the present study, 40.0% of patients with gingival inflammation underwent scaling and/or professional brushing. Previous research emphasized that regular professional hygiene sessions and patient motivation significantly contribute to maintaining periodontal health during orthodontic treatment.[23] Fixed orthodontic treatment also influences adult patients' quality of life. The OHIP-5 assesses key domains of oral health-related quality of life (OHRQoL), including functional limitation, physical pain, psychological discomfort, physical disability, and social disability.[24] This instrument has been validated in several countries, including Romania and Poland.[25,26]

Incorporating OHIP-5-based questions into our questionnaire allowed us to evaluate these aspects. Most participants (81.2%) stated that they often have difficulties chewing, while 40.6% occasionally experienced reduced taste perception following orthodontic treatment. Pain related to orthodontic treatment was commonly reported (57%), suggesting a potential correlation with the orthodontic apparatus activation sessions.

Patients who responded to our questionnaire indicated that they never (30.9%) or hardly ever (23%) felt insecure about the appearance of their mouth due to the orthodontic apparatus. Furthermore, the orthodontic treatment rarely (32.1%) impeded their ability to perform their usual duties. These results demonstrate that patients are focused on the ultimate outcome of treatment and are highly motivated to achieve it.

Finally, emerging evidence indicates that the COVID-19 pandemic has influenced various aspects of oral health, including implant stability, overdenture performance, oral-facial-digital syndrome (OFD), temporomandibular disc displacement, and gingival crevicular fluid and periodontal ligament.[27–32]

Limitations

This study has several limitations. First, the distribution of male and female participants could not be controlled. However, the inclusion of participants of both sexes represents a strength, as it allows the results to be applied to a broader population.

Second, due to restrictions related to the COVID-19 pandemic, the information obtained in the questionnaire could not be correlated with a clinical examination.

Conclusions

Early diagnosis of periodontal conditions during orthodontic treatment is essential for successful therapeutic outcomes. Many adult patients undergoing orthodontic therapy perceive monthly check-ups as sufficient for managing their oral and periodontal needs. However, orthodontists play a central role in educating patients about effective oral hygiene practices, identifying changes of periodontal status, and referring patients for periodontal consultation when needed. The inclusion of periodontal examinations before, during and after orthodontic treatment can lead to the prognosis improvement and reduce the incidence of treatment-related periodontal lesions.

Orthodontic treatment in adults contributes not only to esthetic and functional improvements but also to enhanced psychosocial well-being and overall quality of life.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Gknatidis N, Christou P, Topouzelis N. The orthodontic–periodontic interrelationship in integrated treatment challenges: A systematic review. *J Oral Rehabil*. 2010;37(5):377–390. doi:10.1111/j.1365-2842.2010.02068.x

2. Petrauskiene S, Wanczewska N, Slabsinskiene E, Zemgulyte G. Self-reported changes in oral hygiene habits among adolescents receiving orthodontic treatment. *Dent J (Basel)*. 2019;7(4):96. doi:10.3390/dj7040096
3. Antezack A, Monnet-Corti V. Oral and periodontal hygiene in orthodontic patients [in French]. *Orthod Fr*. 2018;89(2):181–190. doi:10.1051/orthodfr/2018015
4. Levin L, Samorodnitzky-Naveh GR, Machtei EE. The association of orthodontic treatment and fixed retainers with gingival health. *J Periodontol*. 2008;79(11):2087–2092. doi:10.1902/jop.2008.080128
5. Tonetti MS, Mombelli A. Aggressive periodontitis. In: Lindhe J, Lang NP, Karring T, eds. *Clinical Periodontology and Implant Dentistry*. 5th ed. Oxford, UK: Blackwell; 2008:428–457.
6. Saruttichart T, Chantarawaratit PO, Leevailoj C, Thanyasrisung P, Pitiphat W, Matangkasombut O. Effectiveness of a motionless ultrasonic toothbrush in reducing plaque and gingival inflammation in patients with fixed orthodontic appliances. *Angle Orthod*. 2017;87(2):279–285. doi:10.2319/042516-334.1
7. Pandey V, Chandra S, Kumar HPD, Gupta A, Bhandari PP, Rathod P. Impact of dental neglect score on oral health among patients receiving fixed orthodontic treatment: A cross-sectional study. *J Int Soc Prev Community Dent*. 2016;6(2):120–124. doi:10.4103/2231-0762.178752
8. Burlibaşa L, Mocuţa D, Şteţiu AA, et al. The quality of life – major resource of family health. *Eur J Sci Theol*. 2014;10(4):59–67. https://www.ejst.tuiasi.ro/Files/46/6_Burlibasa%20et%20al.pdf. Accessed November 5, 2021.
9. Nassar PO, Bombardelli CG, Schmitt Walker C, et al. Periodontal evaluation of different toothbrushing techniques in patients with fixed orthodontic appliances. *Dental Press J Orthod*. 2013;18(1):76–80. doi:10.1590/s2176-94512013000100017
10. Zhao L, Wang XY, Xu Y, Meng S. Relationship of orthodontic treatment and periodontal soft tissue health [in Chinese]. *Hua Xi Kou Qiang Yi Xue Za Zhi*. 2018;36(6):595–601. doi:10.7518/hxkq.2018.06.003
11. Stoica AM, Steţiu AA, Buruian M, Steţiu M, Monea M. A comparison study between cone-beam computed tomography (CBCT) and regular radiography in endodontics. *J Optoelectron Adv M*. 2016;18(5–6):562–566. https://joam.inoe.ro/articles/a-comparison-study-between-cone-beam-computed-tomography-cbct-and-regular-radiography-in-endodontics. Accessed November 5, 2021.
12. Bock NC, von Bremen J, Kraft M, Ruf S. Plaque control effectiveness and handling of interdental brushes during multibracket treatment – a randomized clinical trial. *Eur J Orthod*. 2010;32(4):408–413. doi:10.1093/ejo/cjp088
13. Yaacob M, Worthington HV, Deacon SA, et al. Powered versus manual toothbrushing for oral health. *Cochrane Database Syst Rev*. 2014;2014(6):CD002281. doi:10.1002/14651858.CD002281.pub3
14. Kurtz B, Reise M, Klukowska M, Grender JM, Timm H, Sigusch BW. A randomized clinical trial comparing plaque removal efficacy of an oscillating-rotating power toothbrush to a manual toothbrush by multiple examiners. *Int J Dent Hyg*. 2016;14(4):278–283. doi:10.1111/idh.12225
15. Quaranta A, Marchisio O, D'isidoro O, Genovesi AM, Covani U. Single-blind randomized clinical trial on the efficacy of an interdental cleaning device in orthodontic patients. *Minerva Stomatol*. 2018;67(4):141–147. doi:10.23736/S0026-4970.18.04104-3
16. Fiorillo L. Chlorhexidine gel use in the oral district: A systematic review. *Gels*. 2019;5(2):31. doi:10.3390/gels5020031
17. Mazzoleni S, De Stefani A, Bordin C, Balasso P, Bruno G, Gracco A. Dental water jet efficacy in the plaque control of orthodontic patients wearing fixed appliance: A randomized controlled trial. *J Clin Exp Dent*. 2019;11(11):e957–e963. doi:10.4317/jced.55411
18. Elkerbout TA, Slot DE, Rosema NAM, Van der Weijden GA. How effective is a powered toothbrush as compared to a manual toothbrush? A systematic review and meta-analysis of single brushing exercises. *Int J Dent Hyg*. 2020;18(1):17–26. doi:10.1111/idh.12401
19. Gallob J, Mateo LR, Chaknis P, Morrison BM, Panagakos F. Randomized controlled trial comparing a powered toothbrush with distinct multi-directional cleaning action to a manual flat trim toothbrush. *Am J Dent*. 2015;28(6):351–356. PMID:26846042.
20. Thienpont V, Dermaut LR, Van Maele G. Comparative study of 2 electric and 2 manual toothbrushes in patients with fixed orthodontic appliances. *Am J Orthod Dentofacial Orthop*. 2001;120(4):353–360. doi:10.1067/mod.2001.116402
21. Ng E, Lim LP. An overview of different interdental cleaning aids and their effectiveness. *Dent J (Basel)*. 2019;7(2):56. doi:10.3390/dj7020056
22. Verrusio C, Iorio-Siciliano V, Blasi A, Leuci S, Adamo D, Nicolò M. The effect of orthodontic treatment on periodontal tissue inflammation: A systematic review. *Quintessence Int*. 2018;49(1):69–77. doi:10.3290/j.qi.a39225
23. Migliorati M, Isaia L, Cassaro A, et al. Efficacy of professional hygiene and prophylaxis on preventing plaque increase in orthodontic patients with multibracket appliances: A systematic review. *Eur J Orthod*. 2015;37(3):297–307. doi:10.1093/ejo/cju044
24. Naik A, John MT, Kohli N, Self K, Flynn P. Validation of the English-language version of 5-item Oral Health Impact Profile. *J Prosthodont Res*. 2016;60(2):85–91. doi:10.1016/j.jpor.2015.12.003
25. Slusanschi O, Moraru R, Garneata L, Mircescu G, Cuculescu M, Preoteasa E. Validation of a Romanian version of the short form of the Oral Health Impact Profile (OHIP-14) for use in an urban adult population. *Oral Health Prev Dent*. 2013;11(3):235–242. doi:10.3290/j.ohpd.a30166
26. Skośkiewicz-Malinowska K, Kaczmarek U, Ziętek M, Malicka B. Validation of the Polish version of the Oral Health Impact Profile-14. *Adv Clin Exp Med*. 2015;24(1):129–137. doi:10.17219/acem/35476
27. Antonelli A, Bennardo F, Brancaccio Y, et al. Can bone compaction improve primary implant stability? An in vitro comparative study with osseodensification technique. *Appl Sci*. 2020;10(23):8623. doi:10.3390/app10238623
28. Minervini G, Romano A, Petruzzi M, et al. Telescopic overdenture on natural teeth: Prosthetic rehabilitation on (OFD) syndromic patient and a review on available literature. *J Biol Regul Homeost Agents*. 2018;32(2 Suppl 1):131–134. PMID:29460531.
29. Minervini G, Romano A, Petruzzi M, et al. Oral-facial-digital syndrome (OFD): 31-year follow-up management and monitoring. *J Biol Regul Homeost Agents*. 2018;32(2 Suppl 1):127–130. PMID:29460530.
30. Moccia S, Nucci L, Spagnuolo C, d'Apuzzo F, Piancino MG, Minervini G. Polyphenols as potential agents in the management of temporomandibular disorders. *Appl Sci*. 2020;10(15):5305. doi:10.3390/app10155305
31. Minervini G, Nucci L, Lanza A, Femiano F, Contaldo M, Grassia V. Temporomandibular disc displacement with reduction treated with anterior repositioning splint: A 2-year clinical and magnetic resonance imaging (MRI) follow-up. *J Biol Regul Homeost Agents*. 2020;34(1 Suppl 1):151–160. PMID:32064850.
32. d'Apuzzo F, Nucci L, Delfino I, et al. Application of vibrational spectroscopies in the qualitative analysis of gingival crevicular fluid and periodontal ligament during orthodontic tooth movement. *J Clin Med*. 2021;10(7):1405. doi:10.3390/jcm10071405

Original paper

Assessment of mandibular canal proximity to molar root apices in a Turkish subpopulation: A cone-beam computed tomography study

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Abstract

Background. The close anatomical relationship between root apices and the mandibular canal (MC) may be of clinical importance in preventing inferior alveolar nerve (IAN) injury during the root canal treatment (RCT) of mandibular molars.

Objectives. The aim of the present study was to evaluate the impact of the mean distance between MC and the root apices of mandibular first and second molars on the risk of IAN injury during RCT in a Turkish subpopulation.

Material and methods. Cone-beam computed tomography (CBCT) images of 934 patients were evaluated. Mandibular molars were examined, and parasagittal sections were used to measure the shortest linear distance between the root apex and the superior cortical border of MC.

Results. The mesial and distal roots were closer to MC in the 18–25-year age group and in females ($p < 0.05$). Overall, 284 (10.5%) mesial roots were in intimate contact with or invading MC, and 80 (3.0%) were located very close to MC (<1.00 mm). Among distal roots, 328 (12.2%) were in intimate contact with or invading MC, 76 (2.8%) were very close to MC, and 2,288 (85.0%) were distant from MC.

Conclusions. The risk of IAN injury during RCT was higher for the distal roots of second molar teeth, especially in the age group of 18–25 years and in female patients.

Keywords: root canal treatment, inferior alveolar nerve, cone-beam computed tomography, mandibular canal, nerve injury

Cite as
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Highlights

- The distal roots of mandibular second molars showed the closest proximity to the mandibular canal (MC), representing the highest potential risk for inferior alveolar nerve (IAN) injury during root canal treatment (RCT).
- Root apices were significantly closer to MC in younger patients (18–25 years) and in females, indicating an increased anatomical risk in these groups.
- A notable proportion of roots were in intimate contact with or invading MC, highlighting the importance of careful working length determination and the use of cone-beam computed tomography (CBCT) in high-risk cases.

Introduction

The mandibular canal (MC) extends from the mandibular foramen to the mental foramen and contains the inferior alveolar artery, inferior alveolar vein, and inferior alveolar nerve (IAN).[1] Paresthesia, dysesthesia, anesthesia, pain, and lip numbness may occur following IAN injury.[2,3] In a study investigating IAN neurosensory disturbances, 19.6% of injuries occurred during dental procedures, and 35.3% of these were reported after endodontic treatment.[4] To prevent such complications, extreme caution is required during root canal treatment (RCT) and surgical procedures in the posterior mandible because of the close anatomical relationship between the superior border of MC and the apices of mandibular molars.[5] Additionally, reduced microarchitectural bone density may create an unimpeded pathway for mechanical and chemical irritants to pass from the root apex to IAN during RCT.[6]

During non-surgical RCT, temporary or permanent IAN injury may occur as a result of over-instrumentation, inaccurate working length determination, the extrusion of irrigating solutions, or the over-extension of root canal obturation materials.[7–11] Root canal sealers, which may exhibit neurotoxic properties before setting, can also contribute to nerve injury.[12,13] In addition, intracanal medicaments may cause nerve damage, and materials such as gutta-percha and paper points have been reported to injure IAN through direct mechanical contact.[14] Furthermore, thermal injury may occur when warm obturation techniques are employed.[15] To minimize these adverse effects and reduce the risk of IAN injury, meticulous care must be taken to avoid the extrusion of materials beyond the apical foramen during RCT.

Cone-beam computed tomography (CBCT) provides three-dimensional (3D) imaging with a moderate radiation dose.[16] Numerous studies have demonstrated the value of CBCT in evaluating relationships among anatomical structures.[17,18] A limited number of investigations have specifically assessed the spatial relationship between root apices and IAN.[19–21] Additionally, certain anatomical variations – such as C-shaped canals, dens invaginatus, radix entomolaris, and talon cusps – have been reported to show ethnic predilections.[22–26] Therefore, variations in the anatomical course and position of IAN may also be expected among different ethnic populations.

The aim of this study was to assess the mean distance between MC and the root apices of mandibular first and second molars in a Turkish subpopulation. The close anatomical relationship between root apices and MC may be of clinical importance in preventing IAN injury during the RCT of mandibular molars.

Material and methods

This study was approved by the ethics committee of Biruni University, Istanbul, Turkey (2020/36-16). In total, 1,244 CBCT scans obtained from patients between January 2019 and December 2019, and saved in the database of the Department of Oral and Maxillofacial Radiology were evaluated.

Eligibility criteria

The inclusion criteria were the presence of at least one fully erupted molar with completely formed roots. The exclusion criteria comprised molars with open apices or severe external root resorption; anatomical variations, such as fusion, taurodontism or radix entomolaris; and CBCT images with artifacts that precluded the accurate visualization of MC or measurement of the distance between root apices and MC.

In total, 934 CBCT scans met the inclusion criteria. The age and sex of the participants were also recorded from the patient files.

Image assessment

A GALILEOS® Comfort Plus CBCT unit (Dentsply Sirona, Bensheim, Germany) was used to acquire the volumetric data, and the images were analyzed using the Sidexis software (Dentsply Sirona). The imaging parameters were as follows: voxel size – 0.25 mm; tube voltage – 98 kV; and field of view (FoV) – 15 cm.

The CBCT images were evaluated independently by a radiologist and an endodontist, each with at least 5 years of clinical experience. The course of MC was manually delineated, and measurements were performed by both

examiners. As the diameter of MC may vary, the software canal-marking tool, which applies a fixed diameter, was used for all CBCT examinations to ensure standardization. The same CBCT unit and software system were used throughout the study.

Prior to the main assessment, the examiners underwent calibration using a set of randomly selected CBCT scans (N = 70) that were not included in the study sample. Following calibration, all images were evaluated, and any discrepancies were resolved by discussion until consensus was reached. The obtained Cohen's kappa coefficient indicated very good inter-observer agreement (κ = 0.87).[27] All evaluations were performed on an Asus Pro A4310-BB158M All in One PC (ASUSTeK Computer Inc., Taipei, Taiwan) equipped with a high-definition, wide-screen LED monitor (resolution: 1,600 × 900 pixels) under dim ambient lighting conditions.

All molar teeth were evaluated in 3 planes (sagittal, axial and coronal) to determine the shortest linear distance between the root apex and the superior cortical border of MC (Fig. 1). Distances <1.00 mm were classified as very close to MC (Fig. 2).[28] Distances ≥1.00 mm were considered distant from MC (Fig. 3).[28] When the root apex was in intimate contact with or invading MC, the distance was recorded as 0 mm (Fig. 4).

The patients were categorized into the following age groups: 18–25 years; 26–35 years; 36–45 years; 46–55 years; 56–65 years; and >65 years. The potential effects of age and sex were subsequently analyzed.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA). The normality of the data was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests, which indicated that the parameters were normally distributed. Qualitative data were compared using the one-sample χ^2 test. The relationships between the distance from root apices to MC and age or sex were analyzed using Student's t test for independent samples. One-way analysis of variance (ANOVA) was employed to compare parameters across multiple age groups. For comparisons between mesial and distal root apices, the paired-samples t test was used. Statistical significance was set at $p < 0.05$.

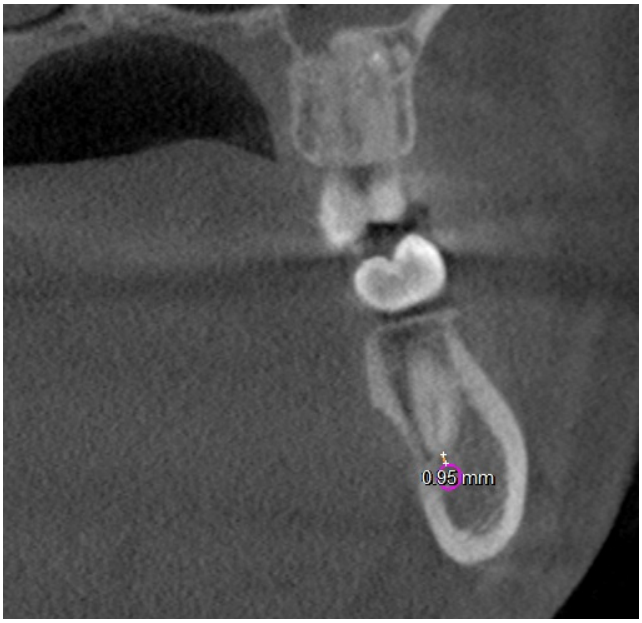

Fig. 2. CBCT image of the coronal section of the right mandibular second molar apex very close (<1.00 mm) to the mandibular canal (MC)

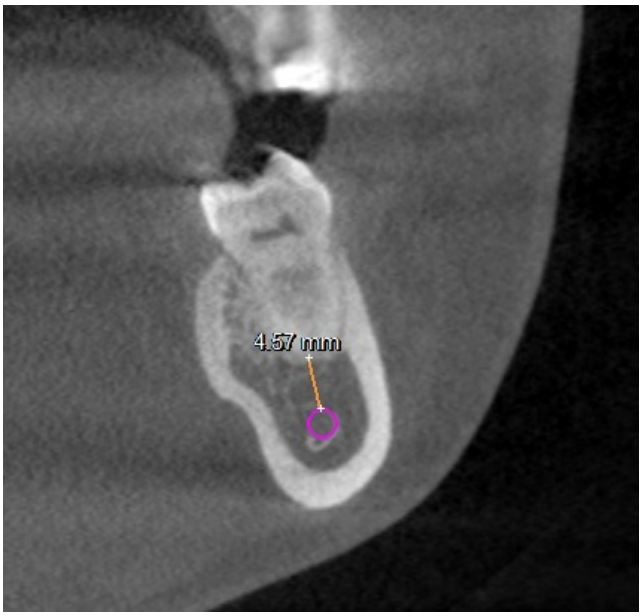

Fig. 3. CBCT image of the coronal section of the right mandibular first molar apex distant (≥1.00 mm) from the mandibular canal (MC)

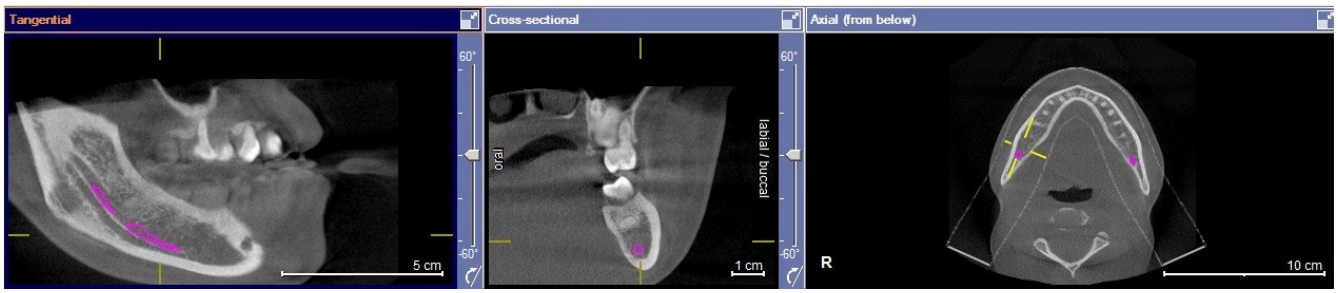

Fig. 1. Exemplary cone-beam computed tomography (CBCT) scan showing the panoramic view and the parasagittal sections

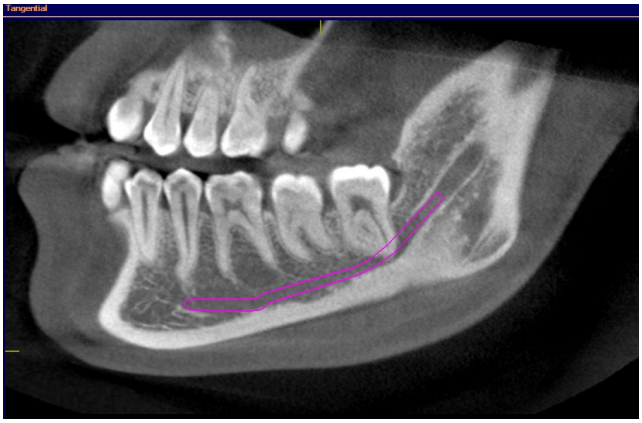

Fig. 4. CBCT image of the sagittal section of the left mandibular second molar roots invading the mandibular canal (MC)

Results

In total, 934 CBCT scans were examined from 442 men (47.3%) and 492 women (52.7%). The patients' age ranged from 18 to 76 years, with a mean of 37.7 +13.6 years. In total, 1,162 first and 1,530 second molar teeth (5,384 roots of 2,692 teeth) were evaluated. The distances between root apices and MC according to sex and tooth type are shown in Table 1.

In terms of the mean distance, mesial and distal roots were closer to MC in the 18–25-year age group as compared to other age groups ($p < 0.05$). Mesial roots in the 26–35-year age group were significantly closer to MC than in the 46–55-year age group ($p < 0.05$). Similarly, distal roots in the 26–35-year age group were significantly closer to MC than in the 46–55- and 56–65-year age groups ($p < 0.05$). In addition, in terms of both mesial and distal roots, the mean distance in the 36–45-year age group was significantly shorter as compared to the 46–55-year age group ($p < 0.05$) (Table 2, Fig. 5). With respect to sex, both mesial and distal root apices were significantly closer to MC in females than in males ($p < 0.05$) (Table 2).

Overall, 284 (10.5%) mesial roots were in intimate contact with or invading MC, 80 (3.0%) were very close to MC, and 2,328 (86.5%) were distant from MC. A total of 328 (12.2%) distal roots were in intimate contact with or invading MC, 76 (2.8%) were very close to MC, and 2,288 (85.0%) were distant from MC (Table 3).

In the comparison of the mean distances of the mesial and distal root apices of first and second molars to MC, the distal roots of first and second molars were closer to MC than mesial roots ($p < 0.05$) (Table 4).

Table 2. Mean distances between root apices and the mandibular canal (MC) for all teeth included according to age group and sex [mm]

Variable		Mesial root	Distal root
Age group [years]	<25	3.16 ±2.51	2.80 ±2.46
	26–35	4.46 ±2.96	4.16 ±2.82
	36–45	4.85 ±2.82	4.43 ±2.73
	46–55	5.20 ±2.92	5.00 ±2.87
	56–65	4.86 ±3.00	4.95 ±3.04
	>65	5.58 ±1.85	5.36 ±3.02
p-value[1]		0.000*	0.000*
Sex	M	4.96 ±3.08	4.62 ±3.02
	F	3.62 ±2.54	3.35 ±2.53
p-value[2]		0.000*	0.000*

Data presented as mean ± standard deviation (*M* ±*SD*).
[1] one-way ANOVA; [2] Student's *t* test; * statistically significant ($p < 0.05$).

Table 1. Distances between root apices and the mandibular canal (MC) according to sex and tooth type [mm]

Tooth	Sex	Mesial root				Distal root			
		n (%)	min	max	*M* ±*SD*	*n* (%)	min	max	*M* ±*SD*
Left first molar	M	282 (10.5)	0.00	13.03	5.51 ±3.07	282 (10.5)	0.00	12.97	5.23 ±3.06
	F	310 (11.5)	0.00	13.80	4.23 ±2.35	310 (11.5)	0.00	14.01	3.98 ±2.48
	total	592 (22.0)	0.00	13.80	4.84 ±2.79	592 (22.0)	0.00	14.01	4.56 ±2.84
Left second molar	M	374 (13.9)	0.00	15.66	4.51 ±3.08	374 (13.9)	0.00	15.44	4.15 ±2.91
	F	398 (14.8)	0.00	10.54	2.94 ±2.40	398 (14.8)	0.00	10.62	2.81 ±2.40
	total	772 (28.7)	0.00	15.66	3.70 ±2.86	772 (28.7)	0.00	15.44	3.46 ±2.74
Right first molar	M	282 (10.5)	0.00	13.50	5.79 ±3.00	282 (10.5)	0.00	13.16	5.53 ±2.98
	F	288 (10.7)	0.00	13.14	4.59 ±2.53	288 (10.7)	0.00	12.69	4.20 ±2.68
	total	570 (21.2)	0.00	13.50	5.18 ±2.83	570 (21.2)	0.00	13.16	4.85 ±2.90
Right second molar	M	366 (13.6)	0.00	12.51	4.36 ±2.95	366 (13.6)	0.00	11.26	3.94 ±2.88
	F	392 (14.6)	0.00	12.32	3.09 ±2.48	392 (14.6)	0.00	10.25	2.79 ±2.28
	total	758 (28.2)	0.00	12.51	3.70 ±2.79	758 (28.2)	0.00	11.26	3.35 ±2.65
Total	M	1,304 (48.4)	0.00	15.66	4.96 ±3.08	1,304 (48.4)	0.00	15.44	4.62 ±3.02
	F	1,388 (51.6)	0.00	13.80	3.61 ±2.53	1,388 (51.6)	0.00	14.01	3.35 ±2.53
	total	2,692 (100.0)	0.00	15.66	4.26 ±2.89	2,692 (100.0)	0.00	15.44	3.97 ±2.85

M – male; F – female; min – minimum; max – maximum; *M* – mean; *SD* – standard deviation.

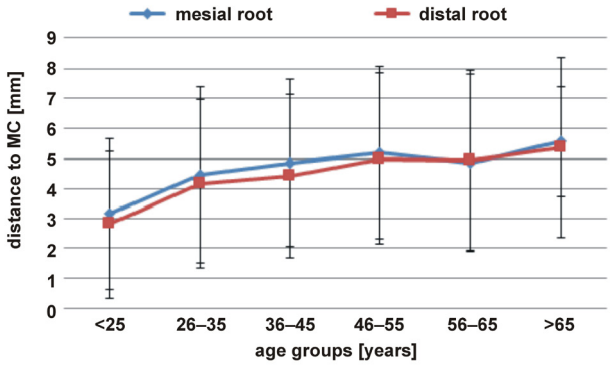

Fig. 5. Distances between mesial and distal root apices and the mandibular canal (MC) for different age groups

Discussion

Only a limited number of studies have investigated the relationship between root apices and MC from an endodontic perspective, using CBCT.[29,30] These studies measured the distance between root apices and MC in mandibular molars, and reported that the distal roots of second molars were in the most intimate contact with MC, followed by the mesial roots of second molars.[29,30] Kim et al. previously confirmed the reliability and accuracy of CBCT for measuring the proximity between anatomical structures.[31] Therefore, the present study utilized CBCT images to evaluate the relationship between the root apices of first and second molars and MC in a selected subpopulation.

In the current study, the shortest distance between root apices and MC was observed in the 18–25-year age group. Similarly, Bürklein et al. reported that the distance between MC and root apices increases with age.[21] These findings may be attributed to the continued growth of the facial skeleton during young adulthood.[21,28,31–34]

Consistent with several previous studies,[21,35] the present results demonstrated shorter distances between root apices and MC in females as compared to males. This finding suggests that the risk of IAN damage may be greater in females than in males.[8,36] Females generally have smaller body dimensions than males, which may account for the reduced distance between root apices and MC observed in women.

Table 4. Comparison of the mean distances of the mesial and distal root apices of first and second molars to the mandibular canal (MC)

Tooth	*n*	Mesial root	Distal root	*p*-value
First molar	1,162	5.01 ±2.81	4.71 ±2.87	0.000*
Second molar	1,530	3.70 ±2.82	3.40 ±2.70	0.000*
Total	2,692	4.26 ±2.89	3.97 ±2.85	0.000*

* statistically significant ($p < 0.05$; paired-samples *t* test).

Among the molar teeth examined, MC was located closer to the mesial and distal root apices of second molars than to those of first molars. Furthermore, in both first and second molars, distal roots were closer to MC than mesial roots. These findings are consistent with previous research.[19,20] This anatomical proximity may help explain the higher incidence of paresthesia reported after the endodontic treatment of second molars.[37]

The results of the present study demonstrated that the distal roots of second molars were not only the closest to MC, but also exhibited the highest percentage of intimate contact with or invasion of MC. Additionally, 11.4% of the evaluated mandibular molars had root apices that were in intimate contact with or invading MC. Consistent with these findings, Chong et al.[19] and Oliveira et al.[38] found intimate associations between MC and root apices in more than 8% and 50% of cases, respectively. In contrast, Lvovsky et al. reported that 3.4% of the roots assessed in their CBCT study were in intimate contact with MC.[39] These discrepancies among studies may be attributable to differences in genetic background and ethnicity across the populations examined.

The findings regarding the proximity of root apices to MC have important clinical implications. They indicate that the extrusion of irrigating solutions or filling materials, as well as over-instrumentation, may result in iatrogenic injury to IAN.[40,41] Therefore, the accurate determination of the working length is essential not only to minimize the risk of IAN injury, but also to ensure the success of endodontic treatment, particularly in cases where root apices are in intimate contact with or invade MC. As suggested in previous research, the use of new-generation apex locators in combination with conventional radiographic techniques may provide more accurate working length determination than either method used alone.[42]

Table 3. Distance classifications according to tooth type

Root	Distance classification	Left first molar (*n* = 592)	Left second molar (*n* = 772)	Right first molar (*n* = 570)	Right second molar (*n* = 758)	Total (*N* = 2,692)
Mesial root	intimate contact/invading	34 (5.7)	110 (14.2)	24 (4.2)	116 (15.3)	284 (10.5)
	very close	2 (0.3)	28 (3.6)	22 (3.9)	28 (3.7)	80 (3.0)
	distant	556 (93.9)	634 (82.1)	524 (91.9)	614 (81.0)	2,328 (86.5)
Distal root	intimate contact/invading	32 (5.4)	114 (14.8)	42 (7.4)	140 (18.5)	328 (12.2)
	very close	14 (2.4)	32 (4.1)	10 (1.8)	20 (2.6)	76 (2.8)
	distant	546 (92.2)	626 (81.1)	518 (90.9)	598 (78.9)	2,288 (85.0)

Data presented as number (percentage) (*n* (%)).

Symptoms indicative of IAN injury may include acute pain or paresthesia, and can progress to inflammation and edema.[43,44] Contemporary endodontic practice emphasizes minimally invasive approaches supported by magnification and enhanced illumination systems.[45] These modern techniques may be beneficial in the management of cases involving IAN injury following RCT.[46,47]

In an analysis of 134 CBCT scans, Chong et al. reported that MC was located ≤3 mm from root apices in 54.8% of cases.[19] However, the authors did not explain the rationale for selecting ≤3 mm as the reference threshold. In the present study, a distance of <1 mm was defined as 'very close', based on previous reports indicating that the accuracy of electronic apex locators ranges from 90% to 100% within ±1 mm of the apical foramen.[39,48,49] The recommended technique when using an electronic apex locator is to advance the instrument to the apical foramen until the apex is indicated, and then withdraw it 0.5–1 mm coronally.[50] However, in cases where the root apex is in intimate contact with MC, this approach may increase the risk of nerve injury.

Limitations

Although the risk of IAN injury during RCT is greatest when root apices invade MC, the risk remains considerable in cases of intimate contact. For this reason, the roots invading MC and those in intimate contact with it were categorized together in the present study. This approach may be regarded as a limitation.

One of the inclusion criteria was the presence of at least one molar tooth. However, when the adjacent molar is missing, the remaining molar may undergo mesio-distal inclination over time. Such positional changes could have influenced the measured mean distances, and therefore represent an additional limitation. Future studies may address this issue by including only cases in which both first and second molars are present bilaterally.

Furthermore, factors such as ethnicity and certain systemic medical conditions may influence the risk of IAN injury during RCT. Endodontic procedures should therefore be performed with particular caution in populations reported to have shorter distances between root apices and MC, as well as in patients with medical conditions that may impair healing.

Conclusions

We conclude that the shortest distance between root apices and MC is observed in the distal roots of second molars, particularly in patients aged 18–25 years and in female individuals. Cases in which root apices are in intimate contact with or invade MC are representing a clinically relevant anatomical finding.

CBCT imaging, which clearly demonstrates the relationship between anatomical structures without superimposition, may serve as a valuable tool for identifying high-risk cases and minimizing the likelihood of iatrogenic IAN injury.

Ethics approval and consent to participate

This study was approved by the ethics committee of Biruni University, Istanbul, Turkey (2020/36-16).

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Toth J, Lappin SL. Anatomy, head and neck, mylohyoid muscle. In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2023. PMID:31424877.
2. Robinson PP. Observations on the recovery of sensation following inferior alveolar nerve injuries. *Br J Oral Maxillofac Surg*. 1988;26(3):177–189. doi:10.1016/0266-4356(88)90161-1
3. Renton T, Yilmaz Z. Managing iatrogenic trigeminal nerve injury: A case series and review of the literature. *Int J Oral Maxillofac Surg*. 2012;41(5):629–637. doi:10.1016/j.ijom.2011.11.002
4. Libersa P, Savignat M, Tonnel A. Neurosensory disturbances of the inferior alveolar nerve: A retrospective study of complaints in a 10-year period. *J Oral Maxillofac Surg*. 2007;65(8):1486–1489. doi:10.1016/j.joms.2007.03.023
5. Pogrel MA. Damage to the inferior alveolar nerve as the result of root canal therapy. *J Am Dent Assoc*. 2007;138(1):65–69. doi:10.14219/jada.archive.2007.0022
6. Givol N, Rosen E, Bjørndal L, Taschieri S, Ofec R, Tsesis I. Medico-legal aspects of altered sensation following endodontic treatment: A retrospective case series. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011;112(1):126–131. doi:10.1016/j.tripleo.2011.01.007
7. Mohammadi Z. Endodontics-related paresthesia of the mental and inferior alveolar nerves: An updated review. *J Can Dent Assoc*. 2010;76:a117. PMID:21118633.
8. Rowe AH. Damage to the inferior dental nerve during or following endodontic treatment. *Br Dent J*. 1983;155(9):306–307. doi:10.1038/sj.bdj.4805219
9. Becking AG. Complications in the use of sodium hypochlorite during endodontic treatment. Report of three cases. *Oral Surg Oral Med Oral Pathol*. 1991;71(3):346–348. doi:10.1016/0030-4220(91)90313-2
10. Scolozzi P, Lombardi T, Jaques B. Successful inferior alveolar nerve decompression for dysesthesia following endodontic treatment: Report of 4 cases treated by mandibular sagittal osteotomy. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2004;97(5):625–631. doi:10.1016/S1079210404000502

11. Poveda R, Bagan JV, Diaz Fernández JM, Sanchis JM. Mental nerve paresthesia associated with endodontic paste within the mandibular canal: Report of a case. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;102(5):e46–e49. doi:10.1016/j.tripleo.2006.03.022
12. Shin Y, Roh BD, Kim Y, Kim T, Kim H. Accidental injury of the inferior alveolar nerve due to the extrusion of calcium hydroxide in endodontic treatment: A case report. *Restor Dent Endod*. 2016;41(1):63–67. doi:10.5395/rde.2016.41.1.63
13. Er K, Ayar A, Kalkan OF, Canpolat S, Tasdemir T, Ozan U. Neurotoxicity evaluation of three root canal sealers on cultured rat trigeminal ganglion neurons. *J Clin Exp Dent*. 2017;9(1):e34–e39. doi:10.4317/jced.52901
14. Ahlgren FK, Johannessen AC, Hellem S. Displaced calcium hydroxide paste causing inferior alveolar nerve paraesthesia: Report of a case. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2003;96(6):734–737. doi:10.1016/j.tripleo.2003.08.018
15. Blanas N, Kienle F, Sándor GK. Inferior alveolar nerve injury caused by thermoplastic gutta-percha overextension. *J Can Dent Assoc*. 2004;70(6):384–387. PMID:15175118.
16. Patel S, Durack C, Abella F, et al.; European Society of Endodontology. European Society of Endodontology position statement: The use of CBCT in endodontics. *Int Endod J*. 2014;47(6):502–504. doi:10.1111/iej.12267
17. Temple KE, Schoolfield J, Noujeim ME, Huynh-Ba G, Lasho DJ, Mealey BL. A cone beam computed tomography (CBCT) study of buccal plate thickness of the maxillary and mandibular posterior dentition. *Clin Oral Implants Res*. 2016;27(9):1072–1078. doi:10.1111/clr.12688
18. Tahmasbi M, Jalali P, Nair MK, Barghan S, Nair UP. Prevalence of middle mesial canals and isthmi in the mesial root of mandibular molars: An in vivo cone-beam computed tomographic study. *J Endod*. 2017;43(7):1080–1083. doi:10.1016/j.joen.2017.02.008
19. Chong BS, Quinn A, Pawar RR, Makdissi J, Sidhu SK. The anatomical relationship between the roots of mandibular second molars and the inferior alveolar nerve. *Int Endod J*. 2015;48(6):549–555. doi:10.1111/iej.12348
20. Koivisto T, Chiona D, Milroy LL, McClanahan SB, Ahmad M, Bowles WR. Mandibular canal location: Cone-beam computed tomography examination. *J Endod*. 2016;42(7):1018–1021. doi:10.1016/j.joen.2016.03.004
21. Bürklein S, Grund C, Schäfer E. Relationship between root apices and the mandibular canal: A cone-beam computed tomographic analysis in a German population. *J Endod*. 2015;41(10):1696–1700. doi:10.1016/j.joen.2015.06.016
22. Merz ML, Isaacson RJ, Germane N, Rubenstein LK. Tooth diameters and arch perimeters in a black and a white population. *Am J Orthod Dentofacial Orthop*. 1991;100(1):53–58. doi:10.1016/0889-5406(91)70049-3
23. Yang ZP, Yang SF, Lin YC, Shay JC, Chi CY. C-shaped root canals in mandibular second molars in a Chinese population. *Endod Dent Traumatol*. 1988;4(4):160–163. doi:10.1111/j.1600-9657.1988.tb00315.x
24. Cakici F, Celikoglu F, Arslan H, Topcuoglu HS, Erdogan AS. Assessment of the prevalence and characteristics of dens invaginatus in a sample of Turkish Anatolian population. *Med Oral Patol Oral Cir Bucal*. 2010;15(6):e855–e858. doi:10.4317/medoral.15.e855
25. Shemesh A, Levin A, Katzenell V, et al. Prevalence of 3- and 4-rooted first and second mandibular molars in the Israeli population. *J Endod*. 2015;41(3):338–342. doi:10.1016/j.joen.2014.11.006
26. Gündüz K, Celenk P. Survey of talon cusps in the permanent dentition of a Turkish population. *J Contemp Dent Pract*. 2008;9(5):84–91. PMID:18633473.
27. Altman DG. *Practical Statistics for Medical Research*. London, UK: Chapman and Hall; 1991.
28. Kovisto T, Ahmad M, Bowles WR. Proximity of the mandibular canal to the tooth apex. *J Endod*. 2011;37(3):311–315. doi:10.1016/j.joen.2010.11.030
29. Littner MM, Kaffe I, Tamse A, Dicapua P. Relationship between the apices of the lower molars and mandibular canal – a radiographic study. *Oral Surg Oral Med Oral Pathol*. 1986;62(5):595–602. doi:10.1016/0030-4220(86)90326-9
30. Denio D, Torabinejad M, Bakland LK. Anatomical relationship of the mandibular canal to its surrounding structures in mature mandibles. *J Endod*. 1992;18(4):161–165. doi:10.1016/S0099-2399(06)81411-1
31. Kim TS, Caruso JM, Christensen H, Torabinejad M. A comparison of cone-beam computed tomography and direct measurement in the examination of the mandibular canal and adjacent structures. *J Endod*. 2010;36(7):1191–1194. doi:10.1016/j.joen.2010.03.028
32. Fudalej P, Kokich VG, Leroux B. Determining the cessation of vertical growth of the craniofacial structures to facilitate placement of single-tooth implants. *Am J Orthod Dentofacial Orthop*. 2007;131(4 Suppl):S59–S67. doi:10.1016/j.ajodo.2006.07.022
33. Love RJ, Murray JM, Mamandras AH. Facial growth in males 16 to 20 years of age. *Am J Orthod Dentofacial Orthop*. 1990;97(3):200–206. doi:10.1016/S0889-5406(05)80052-6
34. Foley TF, Mamandras AH. Facial growth in females 14 to 20 years of age. *Am J Orthod Dentofacial Orthop*. 1992;101(3):248–254. doi:10.1016/0889-5406(92)70094-Q
35. Simonton JD, Azevedo B, Schindler WG, Hargreaves KM. Age- and gender-related differences in the position of the inferior alveolar nerve by using cone beam computed tomography. *J Endod*. 2009;35(7):944–949. doi:10.1016/j.joen.2009.04.032
36. Dempf R, Hausamen JE. Lesions of the inferior alveolar nerve arising from endodontic treatment. *Aust Endod J*. 2000;26(2):67–71. doi:10.1111/j.1747-4477.2000.tb00274.x
37. Von Ohle C, ElAyouti A. Neurosensory impairment of the mental nerve as a sequel of periapical periodontitis: Case report and review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;110(4):e84–e89. doi:10.1016/j.tripleo.2010.03.033
38. Oliveira AC, Candeiro GT, Pacheco da Costa FF, Gazzaneo ID, Alves FR, Marques FV. Distance and bone density between the root apex and the mandibular canal: A cone-beam study of 9,202 roots from a Brazilian population. *J Endod*. 2019;45(5):538–542. doi:10.1016/j.joen.2019.01.004
39. Lvovsky A, Bachrach S, Kim HC, et al. Relationship between root apices and the mandibular canal: A cone-beam computed tomographic comparison of 3 populations. *J Endod*. 2018;44(4):555–558. doi:10.1016/j.joen.2017.12.020
40. Renton T. Minimising and managing nerve injuries in dental surgical procedures. *Fac Dental J*. 2011;2(4):164–171. doi:10.1308/204268511X13154691747058
41. Gambarini G, Plotino G, Grande NM, et al. Differential diagnosis of endodontic- related inferior alveolar nerve paraesthesia with cone beam computed tomography: A case report. *Int Endod J*. 2011;44(2):176–181. doi:10.1111/j.1365-2591.2010.01816.x
42. Ali R, Okechukwu NC, Brunton P, Nattress B. An overview of electronic apex locators: Part 2. *Br Dent J*. 2013;214(5):227–231. doi:10.1038/sj.bdj.2013.215
43. Benevides Froes FG, Aguiar Miranda AM, Da Costa Abad E, Riche FN, Pires FR. Non-surgical management of paraesthesia and pain associated with endodontic sealer extrusion into the mandibular canal. *Aust Endod J*. 2009;35(3):183–186. doi:10.1111/j.1747-4477.2009.00163.x
44. Dubový P, Jančálek R, Kubek T. Role of inflammation and cytokines in peripheral nerve regeneration. *Int Rev Neurobiol*. 2013;108:173–206. doi:10.1016/B978-0-12-410499-0.00007-1
45. Tsesis I, Rosen E, Taschieri S, Strauss YT, Ceresoli V, Del Fabbro M. Outcomes of surgical endodontic treatment performed by a modern technique: An updated meta-analysis of the literature. *J Endod*. 2013;39(3):332–339. doi:10.1016/j.joen.2012.11.044
46. Rosen E, Allareddy V, Tsesis I. Case selection for the use of cone beam computed tomography in dentistry based on diagnostic efficacy and risk assessment. In: Rosen E, Nemcovsky CE, Tsesis I, eds. *Evidence-Based Decision Making in Dentistry: Multidisciplinary Management of the Natural Dentition*. Cham, Switzerland: Springer International Publishing; 2017:97–108.
47. Rosen E, Goldberger T, Taschieri S, Del Fabbro M, Tsesis I. Modern surgical endodontic treatment of altered sensation following extrusion of endodontic filling materials. *Refuat Hapeh Vehashinayim (1993)*. 2017;34(1):17–25. PMID:30699491.

48. Aggarwal V, Singla M, Bhasin SS. Influence of instrument size and varying electrical resistance of root canal instruments on accuracy of three electronic root canal length measurement devices. *Int Endod J*. 2017;50(5):506–511. doi:10.1111/iej.12649
49. Connert T, Judenhofer MS, Hülber-J M, et al. Evaluation of the accuracy of nine electronic apex locators by using micro-CT. *Int Endod J*. 2018;51(2):223–232. doi:10.1111/iej.12814
50. Oliveira TN, Vivacqua-Gomes N, Bernardes RA, Vivan RR, Hungaro Duarte MA, Vasconcelos BC. Determination of the accuracy of 5 electronic apex locators in the function of different employment protocols. *J Endod*. 2017;43(10):1663–1667. doi:10.1016/j.joen.2017.03.039

Original paper

Comparison of the effectiveness of photodynamic therapy and topical clobetasol in the treatment of oral lichen planus using fractal dimension analysis and lesion autofluorescence

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Abstract

Background. Oral lichen planus (OLP) is an inflammatory condition of mucous membranes with an immunological basis. To date, no standardized treatment protocol has been established. Another complicating factor in clinical research is the assessment of disease activity and treatment efficacy. Fractal dimension analysis offers an objective method for evaluating mucosal irregularities, making it well suited for assessing OLP lesion morphology and treatment outcomes.

Objectives. The aim of the present study was to assess the effectiveness of photodynamic therapy (PDT) using methylene blue as a photosensitizer and topical clobetasol therapy in the treatment of OLP.

Material and methods. Fractal dimension analysis of oral mucosa images under white light and 405 + 450 nm light was used as an objective method of treatment evaluation.

Results. The use of autofluorescence at 405 + 450 nm enabled the detection of subtle differences in the pattern of the healed mucosa as compared to the reference mucosa, which were not visible during conventional white-light examination. Both treatment methods (topical clobetasol and PDT using methylene blue) demonstrated comparable efficacy in treating OLP.

Conclusions. The use of autofluorescence at 405 + 450 nm is an objective method of assessing healing, more accurate than observations under white light.

Keywords: photodynamic therapy, oral lichen planus, autofluorescence, fractal dimension analysis, clobetasol

Highlights

- This split-mouth randomized controlled trial demonstrated that photodynamic therapy with methylene blue is comparably effective to topical clobetasol in the treatment of erosive oral lichen planus.
- The study utilized polymer-based carriers to enable precise and controlled drug delivery to oral mucosa, thereby minimizing variability associated with conventional application methods.
- Fractal dimension analysis and autofluorescence imaging (405 + 450 nm) were employed as objective assessment tools to evaluate lesion healing and to detect subtle structural changes following treatment.
- Autofluorescence imaging proved superior to conventional white-light examination in identifying structural differences between treated and healthy mucosa.
- The findings support photodynamic therapy as a reliable alternative to topical corticosteroids, with promising potential for the standardization of treatment protocols for oral lichen planus.

Introduction

Oral lichen planus (OLP) is an inflammatory condition of mucous membranes with an immunological basis. The average prevalence of OLP in the global population ranges from 0.89% to 1.01%, varying according to the geographic region, age and gender. It occurs most frequently in Central and South America (1.74%), in individuals over the age of 40, and slightly more often in women.[1,2] The pathomechanism of the disease is not fully understood. The chronic inflammatory response in OLP is triggered by cytotoxic CD8+ and CD4+ T cells and helper T cells, leading to the apoptosis of basal keratinocytes.[3] Interestingly, the proportions of Th17 and Th1 T-cell subtypes may vary depending on the clinical form of OLP, indicating complex pathogenesis.[4] Histologically, a band-like inflammatory infiltrate in the basal layer of the epithelium is observed, composed predominantly of CD8+ T lymphocytes.[5] In addition, the vacuolar degeneration of the basal layer is noted, and the epithelium may appear either acanthotic or atrophic.[6,7] Apart from immune dysregulation, the disease is also believed to involve genetic factors, environmental influences, and infections, particularly with hepatitis C virus (HCV).[5,8–10] Recently, a vitamin D deficiency has also been implicated in increasing the risk of developing OLP.[11,12]

A diagnostic challenge arises from the existence of oral lichenoid lesions (OLLs), including lichenoid contact reactions, drug-induced lichenoid reactions, and the mucosal changes associated with graft-versus-host disease (GvHD). These entities often mimic OLP, both clinically and histologically.[13,14] Nevertheless, bilateral or multifocal distribution remains a key feature supporting the diagnosis of OLP rather than OLL.[15]

Oral lichen planus most commonly presents as asymptomatic, white, reticular lesions located on buccal mucosa and the lateral borders of the tongue. Less commonly observed variants include atrophic, plaque-type, papular, ulcerative, and bullous forms.[16] In these types, patients typically report discomfort, a burning sensation, or pain – either spontaneous or triggered by eating, which negatively impacts quality of life (QoL).[17] From a clinical standpoint, a crucial issue is the potential for malignant transformation, particularly in the erosive and atrophic forms of OLP, with reported rates ranging from 0.04% to 1.74%.[16,18–23] According to some authors, this value may be underestimated due to the lack of standardized diagnostic criteria.[24] Esthetically and functionally, the oral cavity is highly sensitive to both treatment and disease progression. Therefore, managing OLP poses a therapeutic challenge for many clinicians, particularly because treatment is symptomatic rather than curative.

To date, no standardized treatment protocol has been established. Reticular lesions usually do not require intervention and are managed through regular monitoring.[25,26] These lesions are typically asymptomatic, and patients may be unaware of their presence. In contrast, intervention is required for other forms of OLP due to the associated pain and the risk of malignant transformation. The primary therapeutic goal is to eliminate inflammation, thereby reducing pain and promoting lesion remission.[25] Among the available treatment modalities, topical corticosteroids (TCSs) remain the first-line option. Systemic corticosteroids are reserved for advanced, treatment-resistant cases, particularly those involving mucocutaneous or organ involvement (e.g., the esophagus or genitals). These are often combined with other immunosuppressive or biologic agents.[25]

The most commonly used TCSs include triamcinolone acetonide, clobetasol propionate, betamethasone valerate, dexamethasone, and fluocinolone acetonide, which are applied in the form of creams, gels, ointments, or submucosal injections.[27,28] Oral corticosteroids include betamethasone, prednisolone and methylprednisolone.[29,30] Due to the side effects of these drugs and their incomplete efficacy, alternative therapeutic agents are continually explored. These include humanized mono-clonal antibodies (efalizumab), chemotherapeutics (dapson), interferons, retinoids (tretinoin, isotretinoin, tazarotene), calcineurin

inhibitors (cyclosporine, tacrolimus, pimecrolimus, sirolimus), methotrexate, hyaluronic acid, amitriptyline, and amlexanox.[31–35]

Clinicians are increasingly turning to photodynamic therapy (PDT), which has demonstrated efficacy comparable to that of TCSs.[36–38] Photodynamic therapy acts by selectively inducing apoptosis in the proliferating T lymphocytes (CD4+, CD8+, Th17) through the generation of reactive oxygen species (ROS). These ROS are produced when light is absorbed by a photosensitizer that accumulates in hyperproliferative cells. In addition to favorable clinical outcomes, PDT is easy to administer, well tolerated by patients, and exhibits high selectivity at the cellular and molecular levels – advantages not observed with steroid therapy. Furthermore, PDT leads to systemic anti-inflammatory effects, such as reduced plasma levels of CXCL10 chemokine and decreased numbers of circulating CD4+, CD137+, CD8+ cells, and IL-17-secreting T lymphocytes.[39] However, some researchers still question the efficacy of PDT in treating OLP.[40] From a clinical standpoint, PDT is a promising therapeutic approach. However, differences in light source selection, the photosensitizer type, the application method, and the number of treatment sessions may significantly affect outcomes. Further studies are therefore needed in this area.

What is also a significant challenge in OLP management is effective drug delivery. The moist oral environment hinders the retention and therapeutic activity of topical agents. Polymer-based drug delivery systems that adhere to the mucosa and enable controlled release represent a promising innovation.

Another complicating factor in clinical research is the assessment of disease activity and treatment efficacy. Commonly used scales, such as the numerical rating scale (NRS), Thongprasom, Carrozzo and Gandolfo, or the oral lichen planus disease activity scale (OLP-DAS), are not entirely accurate due to the measurement errors associated with mucosal mobility and subjective assessments by the clinician or patient.[41–43] The size of mucosal lesions is influenced by muscle tension, and the irregularity of the affected areas further complicate measurement. Additionally, image detection and evaluation depend on the light source, specifically, the wavelength of the emitted light, which was demonstrated in our previous research.[44] Our evaluation of healing under white light and at 405 + 450 nm combined wavelength reveals differences between these spectral ranges. This approach complements visual assessment and aids clinicians in interpreting treatment outcomes.[44]

From a geometric perspective, classical Euclidean concepts are insufficient for describing the irregular morphology of OLP lesions. Fractal geometry, which allows dimensional values between 0 and 3, provides a more appropriate framework. Complex and irregular two-dimensional (2D) structures exhibit lower fractal dimension (FD) values, whereas simpler shapes approach a dimension of 2. Figure 1 illustrates the contrast between the FD of a square and the Sierpiński carpet. Fractal analysis therefore offers an objective, quantitative method for evaluating mucosal irregularities, making it well suited for assessing OLP lesion morphology and treatment outcomes.

Objectives

The objective of the present study was to compare the therapeutic effectiveness of PDT with methylene blue and topical clobetasol in the management of OLP. Treatment outcomes were evaluated using an objective assessment method based on fractal dimension analysis of oral mucosa images acquired under white light and 405 + 450 nm autofluorescence. An innovative polymer-matrix drug-delivery system was implemented, designed to reduce mucosal contact time for the photosensitizer while extending the contact duration of the corticosteroid.

The following null hypotheses were formulated:

1. There are no statistically significant differences in the lesion surface area before and after treatment.
2. The mean reduction in the lesion surface area before and after treatment is the same for both therapeutic methods.
3. The mean fractal dimension (FD) of the lesions before treatment does not differ significantly from that of healthy oral mucosa, under both white light and at 405 + 450 nm combined wavelength.
4. There are no statistically significant differences between the FD values of the lesions before and after treatment, under white light and at 405 + 450 nm combined wavelength.
5. There are no statistically significant differences in the FD values of the lesions before and after treatment with regard to the treatment method used.
6. There are statistically significant differences in the FD values of the lesions after treatment as compared to healthy oral mucosa under white light.
7. There are statistically significant differences in the FD values of the lesions after treatment as compared to healthy oral mucosa at 405 + 450 nm combined wavelength.

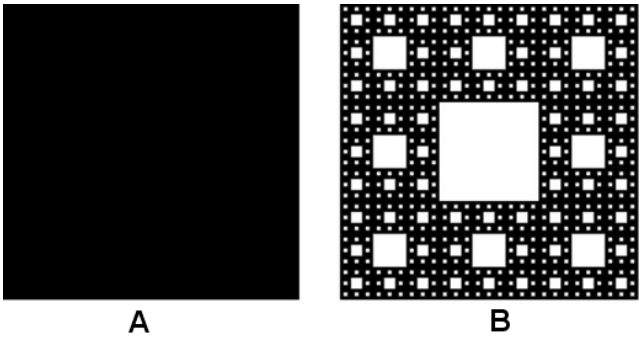

Fig. 1. Fractal dimensions (FD) of a square (FD = 2) (A) and the Sierpiński carpet (FD ≈ 1.8920) (B)

Generated using fractal generator software (https://codinglab.huostravelblog.com/math/fractal-generator).

Material and methods

Design and patients

This project was conducted at the Oral Mucosa and Periodontal Disease Clinic of the Academic Dental Polyclinic at Wroclaw Medical University, Poland. In accordance with the Polish law, it was classified as a therapeutic medical experiment and received prior approval from the Bioethics Committee of Wroclaw Medical University (approval No. kb21/2023n). All participants provided written informed consent before enrollment, in accordance with the Declaration of Helsinki.

Methodologically, the study was designed as a split-mouth randomized controlled trial (RCT) to minimize the influence of interindividual variability on treatment outcomes. The study was retrospectively registered at ClinicalTrials.gov (NCT06752343).

The sample size was determined using McNemar's test formula, assuming a type I error rate of 5%, a power of 0.8, a success rate of 0.75 in both groups, and a failure rate of 0.25.

A total of 29 patients participated in the study. Patients with bilateral erythematous or erosive OLP lesions larger than 1 cm in diameter, confirmed histopathologically at the Department of Clinical Pathology of Wroclaw Medical University, were included in the study. The qualification process and assignment of numbers from 1 to 29 were conducted by the same physician (TPK). The main exclusion criterion was dysplasia identified in the histopathological examination. Other exclusion criteria included diabetes, liver diseases, smoking (including e-cigarettes with nicotine), pregnancy, or the objective determination of a lichen planus-like nature of the lesions. The clinical assessment of the evolution of OLP lesions was performed at baseline, at the end of both forms of treatment (day 12) and after 12 weeks without treatment. Patient workflow is presented in Fig. 2.

Carriers with clobetasol and methylene blue

The method of preparation and the composition of carriers for the active substances used in the present study were described in the authors' initial pilot study.[45] The matrices were obtained by lyophilizing the foam formed from the dispersion of pullulan, sodium alginate, glycerol, and methylcellulose. The active substances – clobetasol (Pol-Aura, Zabrze, Poland) or methylene blue (abcr, Karlsruhe, Germany) – were added to the homogeneous mixtures (Table 1).

The developed carriers containing clobetasol and methylene blue demonstrated high mechanical strength, smear resistance, and effective release of active substances during in vitro tests. The carrier was precisely fitted to the size of the lesion treated on the mucosa, allowing the accurate determination of the active substance amount in mg/cm^2 in the form of a dry carrier.[45]

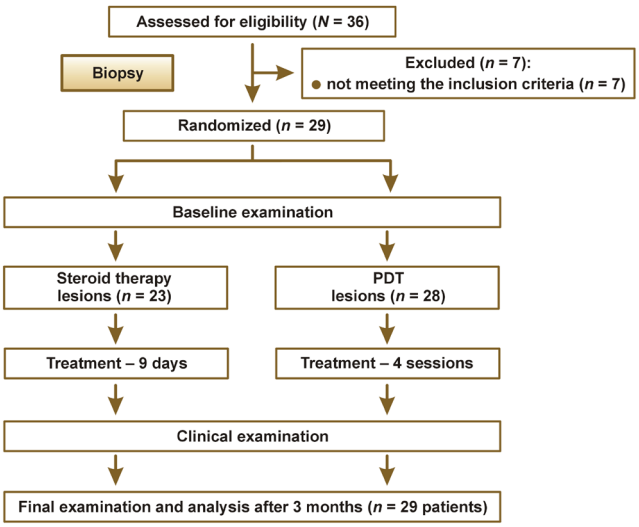

Fig. 2. Patient workflow
PDT – photodynamic therapy.

Table 1. Tytuł tabeli

Carrier for	Percentage in dry mass [%]				
	pullulan	sodium alginate	glycerol	methylcellulose	active substance
Clobetasol	36.60	9.70	48.60	4.85	0.25
Methylene blue	27.00	7.30	38.90	4.30	22.50

Laser device

In our study, we used a 405-nm, 450-nm and 640-nm laser device. This device was invented and manufactured at the Institute of High Pressure Physics, Polish Academy of Sciences, Warsaw, Poland. A reflector in the form of a regular pyramid couples laser diodes into a multimode fiber. This device is able to couple into one optic fiber 2 or 3 wavelengths together.

Photodynamic therapy procedure

The treated lesion was entirely covered for 10 min with the carrier containing methylene blue, and then irradiated with a diode laser with a wavelength of 640 nm, at a dose of 120 J/cm^2 and a power of 520 mW, a spot diameter of 8 mm, power density of 1.034 W/cm^2, and irradiation time 227 s. Photodynamic therapy was repeated in 4 sessions, at 3-day intervals. Each time, the procedure was conducted by the same physician (KJ).

Image acquisition

In previous studies, we demonstrated that the highest level of image detail can be captured from photographs

taken using the autofluorescence of the lesions excited with a combined wavelength of 405 + 450 nm.[44] All photographs were taken using a Canon EOS 77D camera, with a Canon 60 mm f/2.8 EF-S USM Macro lens (Canon Inc., Ota City, Tokyo, Japan), with Metz 15 MS-1 ring light (Metz, Markham, Canada). The same distance (45 cm) was set to take all photos. The optical axis of the camera was kept perpendicular to the surface of the lesion. The focus plane was locked to 45 cm to obtain repeatability. During laser irradiation, photographs were taken in the same way as under white light. Laser parameters were set to 1.6 W for 405 + 450 nm. The optic fiber was equipped with a special diffuser of light, enabling the uniform irradiation of the surface. To achieve similar histogram filling, we used the following parameters of photographs: 405 + 450 nm – ISO3200, f/9, 1/500 s; white light – ISO 3200, f/9, 1/250s. The regions of interest (ROIs) were 300 × 300 pixels in size. They were selected from both healthy mucosa and the pathologically altered areas, each time from the same anatomical structures. For example, if the pathological lesion was located on buccal mucosa, the reference ROI was taken from the unaffected area of buccal mucosa. The ROIs before and after therapy were selected from the same locations by simultaneously analyzing pre- and post-treatment images.

Fractal dimension analysis

The ImageJ software, v. 1.53e (National Institutes of Health (NIH), Bethesda, USA, https://imagej.net/ij), and the FracLac plugin, v. 2.5 (Charles Sturt University, Bathurst, Australia, public domain license) were used to conduct fractal dimension analysis. The procedure of FD calculation was described in detail in our previous study.[44]

Statistical analysis

Statistical analysis was performed using Statistica, v. 13.3 (StatSoft, Krakow, Poland), and 0.05 was set as the statistically significant level. The normality of distribution was determined with the Shapiro–Wilk test. The homogeneity of variance was checked using Levene's test. In case of normal distribution, we performed parametric tests. We applied the paired and unpaired Student's *t* test. If the distribution was not normal, we turned to non-parametric tests – the Wilcoxon and Mann–Whitney *U* tests. The lack of normal distribution was observed for the perimeter (and the associated values, for example the area, etc.) and surface FD.

Results

Table 2 presents the lesion surface area values before and after treatment, categorized by the therapeutic method. Notably, the standard deviation (*SD*) values for the lesion surface area were high both before and after treatment, as well as for the difference between the 2 time points. In the latter case, the *SD* values significantly exceeded the mean (*M*) values. The mean reduction in the lesion surface area was 112.7 ±236.0 mm^2 for steroid therapy and 199.1 ±240.2 mm^2 for PDT. Null hypothesis 1 was rejected.

No statistically significant differences were observed in terms of reduction in the lesion surface area between the treatment methods (Table 3). Null hypothesis 2 was retained.

Table 4 presents the FD of lesions before treatment in comparison with the reference oral mucosa under white light and at a wavelength of 405 + 450 nm. Statistically significant differences were observed for both white light and autofluorescence induced by 405 + 450 nm light. Null hypothesis 3 was rejected.

Table 2. Comparison of the lesion surface area before and after treatment by the therapeutic method

Therapeutic method	Time point	*n*	Lesion surface area [mm^2]				*U*	*Z*	*p*-value
			M ±*SD*	*Me*	mean difference	standard deviation difference			
Steroid therapy	before treatment	23	200.2 ±134.9	150	112.7	236.0	22	3.39	0.00069*
	after treatment		87.5 ±186.3	45					
PDT	before treatment	28	249.4 ±247.1	169	199.1	240.2	15	4.18	0.00003*
	after treatment		50.3 ±60.1	38					

M – mean; *SD* – standard deviation; *Me* – median; * statistically significant (Wilcoxon test).

Table 3. Comparison of the difference in the lesion surface area (before and after treatment) between the therapeutic methods

Therapeutic method	Mean difference [mm^2]	Standard deviation difference [mm^2]	*Me*	Sum of ranks	*U*	*Z*	*p*-value
Steroid therapy	112.7	236.0	100	577	301	–0.38806	0.69797
PDT	199.1	240.2	132	749			

Mann–Whitney *U* test.

Table 5 presents the FD values of the lesions before treatment as compared to their FD after treatment for both therapeutic methods. Statistically significant differences in the image structure of the lesions before and after treatment were observed for both topical steroid application and PDT. Null hypothesis 4 was rejected.

No statistically significant differences in the FD of the lesions before and after treatment were found in relation to the applied treatment method, which highlights their comparable effectiveness (Table 6). Null hypothesis 5 was sustained.

In Table 7, the post-treatment FD values of the lesions are compared with the FD values of the reference (healthy) mucosa. Null hypothesis 6 was rejected. Statistically significant differences were observed between the FD of the lesions after treatment and the FD of the reference mucosa when analyzing the lesion images acquired via autofluorescence. Null hypothesis 7 was sustained.

Discussion

With regard to the local treatment of OLP, the key issue is applying the active substance directly onto oral mucosa. Before the introduction of polymeric matrices, both research outcomes and treatment efficacy assessments were uncertain. The use of medications in the form of ointments, rinses, or sterile gauze pads soaked and applied to lesions introduces a significant confounding factor in clinical trials. Such approaches do not guarantee an adequate drug concentration in the pathological tissue, are burdensome for patients, may result in drug overdosage, and can cause a range of side effects. Consequently, comparing studies that employ different application methods for active substances lacks precision and may bias study results, especially in meta-analyses. Introducing polymeric matrices into clinical use eliminates these confounders and, moreover, enables conducting split-mouth RCTs.

Table 4. Comparison of the mean fractal dimension (FD) values of the lesions before treatment relative to the reference oral mucosa, under white light and in autofluorescense at a wavelength of 405 + 450 nm

FD measurement	*n*	FD			*t*	df	*p*-value
		M ±*SD*	mean difference	standard deviation difference			
Before treatment (white)	51	1.5306 ±0.0854	−0.1013	0.0811	−7.70	50	0.00000*
Control (white)		1.6319 ±0.0406					
Before treatment (UV+B)	51	1.4797 ±0.0737	−0.1274	0.0895	−8.77	50	0.00000*
Control (UV+B)		1.6071 ±0.0568					

white – white light; UV+B – autofluorescence in the 405 and 450 nm range; control – reference oral mucosa; df – degrees of freedom; * statistically significant (paired Student's *t* test).

Table 5. Comparison of the mean fractal dimension (FD) values of the lesions before and after treatment, under white light and in autofluorescence at a wavelength of 405 + 450 nm, for both therapeutic groups

Therapeutic method	FD measurement	*n*	FD			*t*	df	*p*-value
			M ±*SD*	mean difference	standard deviation difference			
Steroid therapy	before treatment (white)	23	1.5199 ±0.0922	−0.0667	0.0972	−2.99	22	0.00779*
	control (white)		1.5866 ±0.0788					
	before treatment (UV+B)		1.4998 ±0.0694	−0.0653	0.1064	−2.67	22	0.01554*
	control (UV+B)		1.5651 ±0.0987					
PDT	before treatment (white)	28	1.5377 ±0.0785	−0.0642	0.0770	−3.73	27	0.00143*
	control (white)		1.6019 ±0.0387					
	before treatment (UV+B)		1.4631 ±0.0738	−0.0937	0.1095	−3.83	27	0.00114*
	control (UV+B)		1.5568 ±0.0734					

* statistically significant (paired Student's *t* test).

Table 6. Comparison of the difference in the fractal dimension (FD) of the lesions (before and after treatment) between the therapeutic methods, under white light and in autofluorescence at a wavelength of 405 + 450 nm

Wavelength	Therapeutic method	*n*	Mean difference	Standard deviation difference	*t*	df	*p*-value
White	steroid therapy	23	−0.0667	0.0972	0.09	22	0.92755
	PDT	28	−0.0642	0.0770		27	
UV+B	steroid therapy	23	−0.0653	0.1064	−0.82	22	0.41597
	PDT	28	−0.0937	0.1095		27	

independent Student's *t* test.

Table 7. Comparison of the mean fractal dimension (FD) values of the lesions after treatment relative to the reference mucosa, under white light and in autofluorescense at a wavelength of 405 + 450 nm, for both therapeutic groups

Therapeutic method	FD measurement	*n*	FD			*t*	df	*p*-value
			M ±*SD*	mean difference	standard deviation difference			
Steroid therapy	after treatment (white)	23	1.6007 ±0.0660	−0.0315	0.0637	−2.10	22	0.05119
	control (white)		1.6322 ±0.0346					
	after treatment (UV+B)		1.5581 ±0.0969	−0.0044	0.0821	−2.27	22	0.03651*
	control (UV+B)		1.6021 ±0.0557					
PDT	after treatment (white)	28	1.5982 ±0.0411	−0.0334	0.0723	−2.07	27	0.05283
	control (white)		1.6316 ±0.0462					
	after treatment (UV+B)		1.5674 ±0.0763	−0.0442	0.0889	−2.22	27	0.03860*
	control (UV+B)		1.6116 ±0.0589					

* statistically significant (paired Student's *t* test).

The scientific value of these studies is very high, as they eliminate issues related to the commonly used mucosal drug delivery methods and exclude unknown disrupting factors found in parallel-group randomized trials.[46] Lesions are treated at similar stages, thereby avoiding period effects. Additionally, fewer patients are needed to achieve comparable treatment outcomes than in parallel-group studies.

We revealed statistically significant differences between white light and autofluorescence induced by 405 + 450 nm light. In both cases, the FD values of the examined lesions were lower as compared to the reference mucosa. By definition of fractal dimension, such results indicate a lower degree of structural organization in the lesion pattern as compared to the healthy mucosa. In the case of white-light examination, the FD values for both the lesions and the control mucosa were higher than those obtained from the images captured using autofluorescence. This leads to the conclusion that the use of autofluorescence in the mentioned wavelength range reveals more details in both the lesion image and healthy oral mucosa. For both white light and induced autofluorescence, the FD of the lesions after treatment was higher than before treatment. This observation is interpreted as an increase in the structural order of the examined lesions. However, no statistically significant differences in the FD of the lesions before and after treatment were found in relation to the applied treatment method, which highlights their comparable effectiveness. In the case of white light analysis, no statistically significant differences were observed between the images of the healed lesions and the reference mucosa. This observation was consistent for both TCS treatment and PDT, showing similar levels of significance. However, interesting results were found when analyzing the lesion images acquired via autofluorescence. In this case, statistically significant differences were observed between the FD of the lesions after treatment and the FD of the reference mucosa. This finding supports the hypothesis of the superiority of autofluorescence over conventional white-light examination. Despite the absence of differences in FD between the treated lesions and healthy mucosa under white light, suggesting full healing, the autofluorescence-based images revealed enough subtle structural characteristics to distinguish them from healthy tissue.

Split-mouth RCTs employing the precise application of active substances onto oral mucosa in treating OLP are extremely rare in the literature. Most typically, they involve the submucosal injection of various active substances, rather than precise mucosal delivery as in our study.[47–49] To our knowledge, only two projects have used adhesive membranes as drug carriers. In one, a biodegradable Rivelin®-CLO membrane was used for local clobetasol delivery; it can adhere to moist oral tissues for an average of 90 min.[50] According to the authors, this solution led to a significant reduction in the OLP lesion size, and patients were able to follow the treatment regimen easily. Since the RCTs compared different steroid concentrations with placebo, it is difficult for us to refer to those results.[50] In the other project, the properties of an electrospun, bilayered mucoadhesive system were reported.[51] It comprises an outer hydrophobic backing layer of polycaprolactone (PCL), and an inner mucoadhesive component made by electrospinning polyvinylpyrrolidone (PVP) and Eudragit® RS100 as fiber-forming polymers. Once loaded with drugs, the system promises enhanced therapeutic efficacy through localized and controlled drug delivery to oral mucosa. The authors suggest this solution is a promising option for treating OLP or recurrent aphthous stomatitis (RAS).[51]

Most RCTs comparing steroid therapy with PDT use ointments or rinses for active ingredient delivery. This approach is impractical for oral use: saliva washes away the active substance, making it difficult to achieve an adequate drug concentration in the tissue. Due to the rich vascularization of oral mucosa, there is also a risk of uncontrolled systemic absorption and general effects. Using polymeric matrices in our study eliminates this issue, giving us full control over drug delivery. Another factor affecting PDT outcomes is the light source, specifically its power, wavelength and energy density, as well as the irra-

diation time, and the number of sessions. It is important to remember that light in the visible and near-infrared spectrum penetrates deeper with longer wavelengths: red light penetrates about 0.5 cm at 630 nm and up to 1.5 cm at 700 nm. For OLP, this depth is less significant, as lesions are superficial. However, selecting an appropriate photosensitizer sensitive to a specific wavelength and achieving a sufficient tissue concentration is crucial. Common photosensitizers used in OLP therapy include 5% methylene blue, toluidine blue (TB), methyl-5-aminolevulinate (MAL), and 5-aminolevulinic acid (5-ALA). Considering the many variables in PDT itself, comparing treatment outcomes without standardization is difficult. Nevertheless, systematic reviews unanimously confirm the efficacy of PDT in symptomatic OLP treatment. Additionally, some studies show the superiority of PDT over TCSs in symptom reduction.[38,52–54] However, the limited number of studies – especially with long follow-up – and the methodological differences in PDT justify the need for further research. A meta-analysis by He et al. indicates higher efficacy of topical ALA vs. methylene blue rinses.[53] This difference likely stems from the short rinse time for methylene blue, typically 5 min, as compared to 30–120 min for ALA, which significantly increases the tissue concentration. The authors of the meta-analysis report a PDT recurrence rate of 1.94%, although with follow-up periods varying widely across the studies (3–48 months).[53] Thus, long-term PDT outcomes in OLP treatment remain not fully elucidated, underscoring the urgent need for prolonged follow-up. In our case, patients remain in the program and continue follow-up visits for oncologic vigilance. When comparing the effectiveness of TCSs and PDT in our study, we found both methods to be equivalent. The meta-analysis by Hu et al. aligns with our findings.[53] Another meta-analysis also reported similar efficacy of PDT and TCSs in symptomatic OLP treatment.[55] The analyzed studies used 5-ALA, TB and methylene blue as photosensitizers, and dexamethasone, betamethasone and Kenalog as steroids. Only one study showed TCSs slightly outperforming PDT, though both methods were deemed effective.[56] That meta-analysis included different light sources (diode lasers and LED lamps), and showed significant variation in the irradiation time (5–30 min) and device parameters. Despite this heterogeneity, the meta-analysis suggests PDT is effective in OLP treatment.[56]

The results of our study concur with meta-analyses. Regarding the reduction of the lesion surface area, we found no statistically significant difference between the two methods. We noted high *SD* values for the surface lesion area, both before and after treatment. Similar conclusions emerged from He et al.'s meta-analysis.[53] The FD of the lesions before and after treatment, for both therapeutic options, also showed significant statistical changes, confirming comparable efficacy (with no statistical difference). Likewise, the FD values captured in white light as well as at 405 + 450 nm before and after treatment support the abovementioned similarity. Notably, in white light, the post-treatment FD values and those for the reference mucosa were higher than those obtained at 405 + 450 nm. Thus, the 405 + 450 nm light reveals more detail, greatly enhancing treatment outcome assessment. Consequently, visual evaluation via autofluorescence is superior to white-light imaging.

Autofluorescence enables the analysis of biochemical changes in pathological cells via fluorophores like tryptophan, collagen and porphyrin, which emit fluorescence under appropriate excitation.[57] Autofluorescence is used to detect oral cancer, particularly early-stage, and potentially malignant lesions. Light-based detection systems exploit the metabolic changes in pathological cells affecting absorption or fluorescence.[58] In autofluorescence, the diseased tissues lose fluorescence due to metabolic disruption and altered fluorophore distribution, appearing darker. The commercial VELscope™ system achieves sensitivity for detecting malignant or potentially malignant lesions at 22–100%, and specificity at 16–100%.[58] However, the literature lacks strong evidence to endorse it as a screening tool for oral cancer.[59] To date, no specific spectral signature has been presented for OLP. A few studies suggest that 320 nm light is optimal for assessing the disrupted collagen variability in OLP.[60,61] This data and our findings suggest that developing OLP diagnostics using autofluorescence is worthwhile, and may be useful in monitoring treatment progression.

One cannot ignore parallel-group RCTs comparing PDT and TCSs, which are far more common than split-mouth RCTs. Yet, to our knowledge, no trials compare clobetasol with PDT in OLP treatment. Additionally, active substance applications usually involve rinsing with a photosensitizer solution or pipetting it on, while steroids are often applied via ointments or rinses – methods diametrically opposite to ours. However, the absence of studies like ours necessitates comparison to other designs. Mostafa et al. compared Kenalog with PDT using methylene blue.[37] The steroid was applied as a thin layer of ointment (Kenacort-A Orabase) thrice daily for 30 min, while 5% methylene blue rinse was followed by 660 nm diode laser irradiation (100–130 mW/cm^2) for 70 s, repeated weekly for 2 months. The results indicated PDT was a better option than topical steroids, with greater pain reduction. Yet, their study had significantly more female subjects and a younger average age than ours.[37] Jajarm et al. compared PDT with TB and dexamethasone in OLP treatment.[56] They applied a TB solution (1 mg/mL) by pipette, irradiated after 10 min with a GaAlAs 630 nm laser (10 mW/cm^2 continuous wave, fluence 1.5 J/cm^2), twice weekly for a month. A steroid rinse (5 mg/5 mL) was administered for 5 min, 4 times daily for a month. The authors reported that both methods were effective, though the mean pain improvement and treatment efficacy favored steroids.[56] In our approach, we used similar

wavelengths, but significantly higher power and fluence, with fewer sessions, making comparisons difficult due to methodological differences. Bakhtiari et al. compared PDT with methylene blue and dexamethasone.[62] They used a 630 nm LED lamp with 7.2–14.4 J/cm^2 fluence, irradiating for 30–120 s in sessions on days 1, 4, 7, and 14. Patients rinsed their mouths with 5% methylene blue for 10 min; a steroid rinse (0.5 mg/5 mL) was used for 2 min, 4 times daily for 2 weeks. They found no significant difference between the methods regarding efficacy. Beyond the different PDT protocol, they included erosive and reticular OLP, which is significantly methodologically different from our study.[62] Mirza et al. conducted a parallel-group RCT comparing PDT, low-level laser therapy (LLLT) and topical steroid therapy.[36] The PDT protocol involved TB (1 mg/mL) pipetted, and irradiated after 10 min with a GaAlAs 630 nm laser at 10 W/cm^2 continuous wave, twice weekly for a month. A dexamethasone rinse (5 mg/5 mL) was applied for 5 min, 4 times daily for a month. Pain reduction was higher for TCS, yet treatment efficacy was statistically greater for PDT.[36] All the 4 studies featured divergent methodologies as compared to ours. Notably, frequent irradiation sessions and self-administered steroid use are burdensome for patients, and increase the risk of complications and missed doses, which may significantly bias results.

A database analysis clearly indicates that PDT is an effective alternative to topical steroid therapy. Given the heterogeneity of studies, it is impossible to objectively compare those described in the literature, especially considering parameter variability, like wavelength, and energy density, and drug application methods. Our proposed treatment protocol, using patented drug carriers, is more precise and paves the way for standardizing OLP therapy. Moreover, evaluating lesions under 405 + 450 nm light enhances the sensitivity of OLP lesion detection and facilitates treatment monitoring. Changes in the FD of the treated mucosa compared to the reference mucosa in the 405 + 450 nm spectrum suggest the presence of subtle post-treatment differences, not detectable under standard white-light examination.

Finally, it is worth mentioning non-pharmacological treatment approaches that utilize laser devices. One is LLLT, which shows long-term analgesic effects (>12 weeks). Interestingly, this effect is not observed in the short term (<12 weeks).[63] Additionally, LLLT reduces pathological lesions, which is confirmed by biopsy. Conversely, another meta-analysis reports limited scientific evidence for the superiority of LLLT over corticosteroids.[62] Nevertheless, LLLT represents a promising OLP treatment option, especially where steroid side effects are unacceptable. The range of laser devices is broad, and clinical trials also employ high-energy lasers emitting various wavelengths. Devices used in OLP treatment include Er:YAG, Nd:YAG, CO_2, Er,Cr:YSGG, Nd:YAG, and diode lasers (630–808 nm).[64,65] Research outcomes for these devices show short-term efficacy, but due to the limited number of studies and differences in the irradiation parameter, caution is advised when interpreting such results.

Study limitations

Capturing reproducible intraoral photographs without surface light reflections – even on dried mucosa – remains a significant challenge. An additional limitation is the difficulty in achieving the uniform illumination of the examined area due to the complex anatomical structure of the oral cavity. The study is further limited by the relatively small number of patients evaluated and the short follow-up period after the completion of active treatment. Each case of red OLP requires careful, long-term clinical monitoring because of its potential risk of malignant transformation.

Conclusions

Both treatment methods demonstrated comparable efficacy in managing OLP lesions. Fractal dimension analysis enabled clear differentiation between the treated lesions and healthy mucosa, revealing statistically significant differences in the FD values before and after treatment under both white light and 405 + 450 nm illumination. Additionally, autofluorescence imaging at 405 + 450 nm detected subtle structural differences between the healed and reference mucosa that were not visible under conventional white-light examination, with statistically significant post-treatment differences in the FD values observed under this wavelength.

Trial registration

The study was retrospectively registered at ClinicalTrials.gov (NCT06752343).

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Bioethics Committee at Wroclaw Medical University, Poland (approval No. kb21/2023n). Written informed consent was obtained from all subjects involved in the study.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Artificial intelligence (AI) was used for improving the English language and for preparing the highlights (ChatGPT).

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References

1. González-Moles MÁ, Warnakulasuriya S, González-Ruiz I, et al. Worldwide prevalence of oral lichen planus: A systematic review and meta-analysis. *Oral Dis*. 2021;27(4):813–828. doi:10.1111/odi.13323
2. Li C, Tang X, Zheng X, et al. Global prevalence and incidence estimates of oral lichen planus: A systematic review and meta-analysis. *JAMA Dermatol*. 2020;156(2):172–181. doi:10.1001/jamadermatol.2019.3797
3. Chen S, Wu X, Yang Y, Xu X, Xiong X, Meng W. Increased pathogenicity and pro-inflammatory capabilities of mucosal-associated invariant T cells involved in oral lichen planus. *BMC Oral Health*. 2024;22;24(1):829. doi:10.1186/s12903-024-04621-y
4. Afzali S, Mohammadisoleimani E, Mansoori Y, et al. The potential roles of Th17 cells in the pathogenesis of oral lichen planus. *Inflamm Res*. 2023;72(7):1513–1524. doi:10.1007/s00011-023-01763-7
5. Tziotzios C, Lee JYW, Brier T, et al. Lichen planus and lichenoid dermatoses: Clinical overview and molecular basis. *J Am Acad Dermatol*. 2018;79(5):789–804. doi:10.1016/j.jaad.2018.02.010
6. Müller S. Oral manifestations of dermatologic disease: A focus on lichenoid lesions. *Head Neck Pathol*. 2011;5(1):36–40. doi:10.1007/s12105-010-0237-8
7. Warnakulasuriya S, Kujan O, Aguirre-Urizar JM, et al. Oral potentially malignant disorders: A consensus report from an international seminar on nomenclature and classification, convened by the WHO Collaborating Centre for Oral Cancer. *Oral Dis*. 2021;27(8):1862–1880. doi:10.1111/odi.13704
8. Lai YR, Pan L, Jiang XK, et al. CCL19^{+} fibroblasts promote tertiary lymphoid structure in oral lichen planus: A retrospective study. *Oral Dis*. 2025;31(7):2191–2205. doi:10.1111/odi.15266
9. Reeve MP, Vehviläinen M, Luo S, et al. Oral and non-oral lichen planus show genetic heterogeneity and differential risk for autoimmune disease and oral cancer. *Am J Hum Genet*. 2024;111(6):1047–1060. doi:10.1016/j.ajhg.2024.04.020
10. Medini T, Besbes A, Nasri W, Abdeljelil NB, Medini J. Management of erosive oral lichen planus associated with chronic hepatitis C. *J Ayub Med Coll Abbottabad*. 2024;36(3):651–656. doi:10.55519/JAMC-03-12370
11. Egido-Moreno S, Valls-Roca-Umbert J, Parra-Moreno FJ, Jané-Salas E, Blanco-Carrión A, López-López J. Association of vitamin D levels and oral lichen planus. Systematic review and meta-analysis. *Med Oral Patol Oral Cir Bucal*. 2024;29(5):e626–e633. doi:10.4317/medoral.26603
12. Motahari P, Azar FP, Ahari MA. Role of vitamin D and vitamin D receptor in oral lichen planus: A systematic review. *Ethiop J Health Sci*. 2020;30(4):615–622. doi:10.4314/ejhs.v30i4.17
13. Eisenberg E. Oral lichen planus: A benign lesion. *J Oral Maxillofac Surg*. 2000;58(11):1278–1285. doi:10.1053/joms.2000.16629
14. Van der Waal I. Oral lichen planus and oral lichenoid lesions; a critical appraisal with emphasis on the diagnostic aspects. *Med Oral Patol Oral Cir Bucal*. 2009;14(7):E310–E314. PMID:19300362.
15. Schlosser BJ. Lichen planus and lichenoid reactions of the oral mucosa. *Dermatol Ther*. 2010;23(3):251–267. doi:10.1111/j.1529-8019.2010.01322.x
16. Eisen D. The clinical features, malignant potential, and systemic associations of oral lichen planus: A study of 723 patients. *J Am Acad Dermatol*. 2002;46(2):207–214. doi:10.1067/mjd.2002.120452
17. Hashemipour MA, Sheikhhoseini S, Afshari Z, Gandjalikhan Nassab AR. The relationship between clinical symptoms of oral lichen planus and quality of life related to oral health. *BMC Oral Health*. 2024;13;24(1):556. doi:10.1186/s12903-024-04326-2
18. Gonzalez-Moles MA, Scully C, Gil-Montoya JA. Oral lichen planus: Controversies surrounding malignant transformation. *Oral Dis*. 2008;14(3):229–243. doi:10.1111/j.1601-0825.2008.01441.x
19. Offen E, Allison JR. What is the malignant transformation potential of oral lichen planus? *Evid Based Dent*. 2022;23(1):36–37. doi:10.1038/s41432-022-0240-4
20. Hasan Aghbari SM, Abushouk AI, Attia A, et al. Malignant transformation of oral lichen planus and oral lichenoid lesions: A meta-analysis of 20,095 patient data. *Oral Oncol*. 2017;68:92–102. doi:10.1016/j.oraloncology.2017.03.012
21. González-Moles MÁ, Ruiz-Ávila I, González-Ruiz L, Ayén Á, Gil-Montoya JA, Ramos-García P. Malignant transformation risk of oral lichen planus: A systematic review and comprehensive meta-analysis. *Oral Oncol*. 2019;96:121–130. doi:10.1016/j.oraloncology.2019.07.012
22. Fitzpatrick SG, Hirsch SA, Gordon SC. The malignant transformation of oral lichen planus and oral lichenoid lesions: A systematic review. *J Am Dent Assoc*. 2014;145(1):45–56. doi:10.14219/jada.2013.10
23. Zawadzka B, Pūrienė A, Rimkevičius A. Malignant transformation of oral lichen planus in labial location in a 23-year-old woman – case report. *Dent Med Probl*. 2015;52(4):512–515. doi:10.17219/dmp/60130
24. González-Moles MÁ, Ramos-García P. Malignant transformation of oral lichen planus: Where are we now? *Med Oral Patol Oral Cir Bucal*. 2025;30(1):e65–e75. doi:10.4317/medoral.26834
25. Olson MA, Rogers RS 3rd, Bruce AJ. Oral lichen planus. *Clin Derm*. 2016;34(4):495–504. doi:10.1016/j.clindermatol.2016.02.023
26. Carrozzo M, Porter S, Mercadante V, Fedele S. Oral lichen planus: A disease or a spectrum of tissue reactions? Types, causes, diagnostic algorithms, prognosis, management strategies. *Periodontol* 2000. 2019;80(1):105–125. doi:10.1111/prd.12260
27. Serafini G, De Biase A, Lamazza L, Mazzucchi G, Lollobrigida M. Efficacy of topical treatments for the management of symptomatic oral lichen planus: A systematic review. *Int J Environ Res Public Health*. 2023;20(2):1202. doi:10.3390/ijerph20021202
28. Alshemmari KA, Alzafiri S, Alajmi M, et al. Intra-lesional corticosteroids versus platelet-rich plasma versus platelet-rich fibrin for the treatment of oral lichen planus: A systematic review and network meta-analysis. *Cureus*. 2024;16(9):e69973. doi:10.7759/cureus.69973
29. Malhotra AK, Khaitan BK, Sethuraman G, Sharma VK. Betamethasone oral mini-pulse therapy compared with topical triamcinolone acetonide (0.1%) paste in oral lichen planus: A randomized comparative study. *J Am Acad Dermatol*. 2008;58(4):596–602. doi:10.1016/j.jaad.2007.11.022
30. Chainani-Wu N, Silverman S Jr., Reingold A, et al. A randomized, placebo-controlled, double-blind clinical trial of curcuminoids in oral lichen planus. *Phytomedicine*. 2007;14(7–8):437–446. doi:10.1016/j.phymed.2007.05.003
31. Andabak-Rogulj A, Vindiš E, Aleksijević LH, et al. Different treatment modalities of oral lichen planus – a narrative review. *Dent J (Basel)*. 2023;11(1):26. doi:10.3390/dj11010026
32. Gupta S, Ghosh S, Gupta S. Interventions for the management of oral lichen planus: A review of the conventional and novel therapies. *Oral Dis*. 2017;23(8):1029–1042. doi:10.1111/odi.12634
33. Lodi G, Manfredi M, Mercadante V, Murphy R, Carrozzo M. Interventions for treating oral lichen planus: Corticosteroid therapies. *Cochrane Database Syst. Rev*. 2020;2(2):CD001168. doi:10.1002/14651858.CD001168.pub3
34. Da Silva EL, De Lima TB, Rados PV, Visioli F. Efficacy of topical non-steroidal immunomodulators in the treatment of oral lichen planus: A systematic review and meta-analysis. *Clin Oral Investig*. 2021;25(9):5149–5169. doi:10.1007/s00784-021-04072-7
35. Krawiecka E, Szponar E, Dorocka-Bobkowska B. Severe oral lichen planus responsive to topical cyclosporine A – case report. *Dent Med Probl*. 2015;52(2):241–245. https://dmp.umw.edu.pl/en/article/2015/52/2/241. Accessed October 3, 2025.

36. Mirza S, Rehman N, Alrahlah A, Alamri WR, Vohra F. Efficacy of photodynamic therapy or low level laser therapy against steroid therapy in the treatment of erosive-atrophic oral lichen planus. *Photodiagnosis Photodyn Ther*. 2018;21:404–408. doi:10.1016/j.pdpdt.2018.02.001
37. Mostafa D, Moussa E, Alnouaem M. Evaluation of photodynamic therapy in treatment of oral erosive lichen planus in comparison with topically applied corticosteroids. *Photodiagnosis Photodyn Ther*. 2017;19:56–66. doi:10.1016/j.pdpdt.2017.04.014
38. Akram Z, Javed F, Hosein M, et al. Photodynamic therapy in the treatment of symptomatic oral lichen planus: A systematic review. *Photodermatol Photoimmunol Photomed*. 2018;34(3):167–174. doi:10.1111/phpp.12371
39. Yao C, Xin H, Liu S, et al. Change and significance of connexin 43 in saliva extracellular vesicles from oral lichen planus patients. *Arch Oral Biol*. 2025;173:106217. doi:10.1016/j.archoralbio.2025.106217
40. Jajarm HH, Asadi R, Bardideh E, Shafaee H, Khazaei Y, Emadzadeh M. The effects of photodynamic and low-level laser therapy for treatment of oral lichen planus – a systematic review and meta-analysis. *Photodiagnosis Photodyn Ther*. 2018;23:254–260. doi:10.1016/j.pdpdt.2018.07.001
41. Thongprasom K, Luangjarmekorn L, Sererat T, Taweesap W. Relative efficacy of fluocinolone acetonide compared with triamcinolone acetonide in treatment of oral lichen planus. *J Oral Pathol Med*. 1992;21(10):456–458. doi:10.1111/j.1600-0714.1992.tb00974.x
42. Carrozzo M, Gandolfo S. The management of oral lichen planus. *Oral Dis*. 1999;5(3):196–205. doi:10.1111/j.1601-0825.1999.tb00301.x
43. Somsak P, Piboonratanakit P, Pongsiriwet S, et al. Development and validation of the Oral Lichen Planus-Disease Activity Scale: A novel tool for comprehensive clinical assessment. *Clin Oral Investig*. 2024;28(9):481. doi:10.1007/s00784-024-05875-0
44. Jurczyszyn K, Trzeciakowski W, Kozakiewicz M, et al. Fractal dimension and texture analysis of lesion autofluorescence in the evaluation of oral lichen planus treatment effectiveness. *Materials (Basel)*. 2021;21;14(18):5448. doi:10.3390/ma14185448
45. Zborowski J, Kida D, Szarwaryn A, et al. A comparison of clinical efficiency of photodynamic therapy and topical corticosteroid in treatment of oral lichen planus: A split-mouth randomised controlled study. *J Clin Med*. 2021;19;10(16):3673. doi:10.3390/jcm10163673
46. Qin D, Hua F, He H, Liang S, Worthington H, Walsh T. Quality of split-mouth trials in dentistry: 1998, 2008, and 2018. *J Dent Res*. 2020;99(13):1453–1460. doi:10.1177/0022034520946025
47. Agha-Hosseini F, Atyabi F, Akbari K, Moosavi MS. Decreased recurrence of symptoms in oral lichen planus with intralesional injection of hyaluronic acid and triamcinolone. *Int J Oral Maxillofac Surg*. 2021;50(12):1643–1648. doi:10.1016/j.ijom.2021.02.028
48. Al-Hallak N, Hamadah O, Mouhamad M, Kujan O. Efficacy of injectable platelet-rich fibrin in the treatment of symptomatic oral lichen planus. *Oral Dis*. 2023;29(5):2256–2264. doi:10.1111/odi.14261
49. Bennardo F, Liborio F, Barone S, et al. Efficacy of platelet-rich fibrin compared with triamcinolone acetonide as injective therapy in the treatment of symptomatic oral lichen planus: A pilot study. *Clin Oral Investig*. 2021;25(6):3747–3755. doi:10.1007/s00784-020-03702-w
50. Brennan MT, Madsen LS, Saunders DP, et al. Efficacy and safety of a novel mucoadhesive clobetasol patch for treatment of erosive oral lichen planus: A phase 2 randomized clinical trial. *J Oral Pathol Med*. 2022;51(1):86–97. doi:10.1111/jop.13270
51. Colley HE, Said Z, Santocildes-Romero ME, et al. Pre-clinical evaluation of novel mucoadhesive bilayer patches for local delivery of clobetasol-17-propionate to the oral mucosa. *Biomaterials*. 2018;178:134–146. doi:10.1016/j.biomaterials.2018.06.009
52. Nagi R, Muthukrishnan A, Rakesh N. Effectiveness of photodynamic therapy (PDT) in the management of symptomatic oral lichen planus – a systematic review. *J Oral Biol Craniofac Res*. 2023;13(2):353–359. doi:10.1016/j.jobcr.2023.03.003
53. He Y, Deng J, Zhao Y, et al. Efficacy evaluation of photodynamic therapy for oral lichen planus: A systematic review and meta-analysis. *BMC Oral Health*. 2020;20(1):302. doi:10.1186/s12903-020-01260-x
54. Wang B, Fan J, Wang L, Chai L. Photobiomodulation therapy/photodynamic therapy versus steroid therapy for oral lichen planus: A systematic review and meta-analysis. *Photobiomodul Photomed Laser Surg*. 2021;39(3):145–154. doi:10.1089/photob.2020.4930
55. Al-Maweri SA, Ashraf S, Kalakonda B, Halboub E, Petro W, AlAizari NA. Efficacy of photodynamic therapy in the treatment of symptomatic oral lichen planus: A systematic review. *J Oral Pathol Med*. 2018;47(4):326–332. doi:10.1111/jop.12684
56. Jajarm HH, Falaki F, Sanatkhani M, Ahmadzadeh M, Ahrari F, Shafaee H. A comparative study of toluidine blue-mediated photodynamic therapy versus topical corticosteroids in the treatment of erosive-atrophic oral lichen planus: A randomized clinical controlled trial. *Lasers Med Sci*. 2015;30(5):1475–1480. doi:10.1007/s10103-014-1694-1
57. Ramesh S, Nazeer SS, Thomas S, Vivek V, Jayasree RS. Optical diagnosis of oral lichen planus: A clinical study on the use of auto-fluorescence spectroscopy combined with multivariate analysis. *Spectrochim Acta A Mol Biomol Spectrosc*. 2021;248:119240. doi:10.1016/j.saa.2020.119240
58. Nagi R, Reddy-Kantharaj YB, Rakesh N, Janardhan-Reddy S, Sahu S. Efficacy of light based detection systems for early detection of oral cancer and oral potentially malignant disorders: Systematic review. *Med Oral Patol Oral Cir Bucal*. 2016;21(4):e447–e455. doi:10.4317/medoral.21104
59. Awan KH, Patil S. Efficacy of autofluorescence imaging as an adjunctive technique for examination and detection of oral potentially malignant disorders: A systematic review. *J Contemp Dent Pract*. 2015;16(9):744–749. doi:10.5005/jp-journals-10024-1751
60. Shaiju SN, Ariya S, Asish R, et al. Habits with killer instincts: In vivo analysis on the severity of oral mucosal alterations using auto-fluorescence spectroscopy. *J Biomed Opt*. 2011;16(8):087006. doi:10.1117/1.3608923
61. Haris PS, Balan A, Jayasree RS, Gupta AK. Autofluorescence spectroscopy for the in vivo evaluation of oral submucous fibrosis. *Photomed. Laser Surg*. 2009;27(5):757–761. doi:10.1089/pho.2008.2355
62. Bakhtiari S, Azari-Marhabi S, Mojahedi SM, Namdari M, Rankohi ZE, Jafari S. Comparing clinical effects of photodynamic therapy as a novel method with topical corticosteroid for treatment of Oral Lichen Planus. *Photodiagnosis Photodyn Ther*. 2017;20:159–164. doi:10.1016/j.pdpdt.2017.06.002
63. Lu C, Mo L, Li X, et al. Effects of low-level light therapy on pain and related lesions in patients with oral lichen planus: A systematic review and meta-analysis. *J Evid Based Dent Pract*. 2025;25(2):102126. doi:10.1016/j.jebdp.2025.102126
64. Liu P, Zhou Q, Bao J, et al. Effect of high-intensity laser therapy and photobiomodulation therapy on oral lichen planus – a systematic review and meta-analysis. *Lasers Med Sci*. 2025;40(1):151. doi:10.1007/s10103-025-04398-8
65. Liu Z, Wang L, Ren S, Sun J. Comparison of the effect of the pulsed Er:YAG laser and topical corticosteroid on erosive oral lichen planus: A randomized clinical controlled trial. *Lasers Med Sci*. 2025;40(1):180. doi:10.1007/s10103-025-04419-6

Original paper

Biocompatibility of NeoPUTTY™ versus TheraCal PT® and TheraCal LC® pulp-capping materials, and tissue reaction following subcutaneous implantation in rats: Histological and molecular investigation

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Abstract

Background. Materials used for vital pulp therapy should support the natural healing and regeneration of the dental pulp. Ideally, these materials should be biocompatible, bioactive, non-toxic, and non-carcinogenic. Although numerous medicaments are currently available on the market, none fully meet all the criteria required for an ideal material. In recent years, calcium silicate-based materials, known for their bioactivity and biocompatibility, have gained widespread use in dental practice.

Objectives. This in vivo study investigated the tissue response and biological characteristics of mineral trioxide aggregate (MTA) – NeoPUTTY™, as well as TheraCal PT® and TheraCal LC®, following subcutaneous implantation in rats.

Material and methods. Twenty adult male albino rats were randomly assigned according to the sacrifice time (2 or 4 weeks) into 2 equal groups, which were further subdivided into 4 subgroups based on the material used. Four incisions were made on the back of each rat to create 4 pockets, into which polyethylene tubes were implanted. Three tubes were filled with biomaterials, while the 4th was left empty as a control. After sacrifice, the samples were analyzed histopathologically, histomorphometrically, and for gene expression of vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6).

Results. Moderate inflammation was the predominant tissue response to NeoPUTTY. TheraCal LC demonstrated the formation of a thick connective tissue capsule with moderate chronic inflammatory cell infiltration. In contrast, TheraCal PT showed a mild inflammatory response and a lower area percentage of collagen fibers in the capsule as compared to NeoPUTTY and TheraCal LC. NeoPUTTY and TheraCal LC were associated with higher VEGF and IL-6 expression levels than those observed in the TheraCal PT and control groups.

Conclusions. TheraCal PT appears to be a promising material for direct pulp capping, owing to its favorable biocompatibility when tested in vivo in comparison with NeoPUTTY and TheraCal LC. These findings may assist clinicians in selecting appropriate materials for direct pulp capping, as TheraCal PT demonstrated greater biocompatibility with respect to its effects on the surrounding tissues.

Keywords: biocompatibility, subcutaneous tissue, TheraCal LC®, TheraCal PT®, NeoPUTTY™

Highlights

- Vital pulp therapy materials should promote pulp healing and regeneration.
- Calcium silicate-based materials are widely used due to their bioactivity and biocompatibility.
- TheraCal PT demonstrated superior in vivo biocompatibility and was associated with a reduced inflammatory response in comparison with NeoPUTTY (mineral trioxide aggregate – MTA) and TheraCal LC.
- TheraCal PT shows potential as a promising material for direct pulp capping.
- Further studies are required to evaluate additional properties before clinical application.

Introduction

Dental caries in children is one of the most prevalent chronic degenerative diseases worldwide.[1] Its progression may result in irreversible damage to the dental pulp, potentially leading to the premature loss of deciduous or permanent teeth. One of the primary goals of pediatric dentistry is to maintain deciduous teeth in an intact and functional state until the eruption of their permanent successors.[2] Vital pulp therapy is commonly used treatment aimed at maintaining the vitality of carious teeth and preserving the health of the supporting tissues, thereby allowing deciduous or permanent teeth to be retained for as long as possible.[3]

Ideally, a pulp-capping material should promote the natural healing and regeneration of the dental pulp. It should be biocompatible, bioactive, non-toxic, and non-carcinogenic. In addition, it should exhibit adequate sealing ability, dimensional stability, an appropriate setting time, and good handling characteristics. Although numerous medicaments are currently available on the market, none fully meet all the criteria required for an ideal pulp-capping material.[4] In recent years, calcium silicate-based materials, known for their bioactivity and biocompatibility, have gained widespread use in pediatric dental practice.[5]

Mineral trioxide aggregate (MTA) is one of calcium silicate-based materials that has been used in clinical practice for more than 2 decades. It has demonstrated high clinical effectiveness as a pulp-capping material owing to its excellent sealing ability, biocompatibility, and bioinductive and regenerative properties.[6] Despite these advantages, MTA has several drawbacks, including a long setting time, poor handling characteristics, high solubility, potential for tooth discoloration, and relatively high cost.[7] Consequently, several other calcium silicate-based materials have been developed to overcome these limitations while maintaining the favorable properties of MTA.

TheraCal LC® is a light-cured, resin-modified, calcium silicate-based cement. It has been reported to stimulate hydroxyapatite precipitation and dentin bridge formation.[8] The material is supplied in a syringe, eliminating the need for mixing and simplifying handling. As compared to MTA, TheraCal LC exhibits lower solubility.[9] Its polymerization is associated with minimal heat generation, which may reduce adverse pulpal effects when used as a pulp-capping material. However, the presence of resin components that may remain partially unpolymerized could potentially have adverse effects on the pulpal tissue, particularly as its interaction with the pulp has not been extensively investigated.[10]

TheraCal PT® is a newer calcium silicate-based material; it is a dual-cured, resin-modified cement. It was introduced as a biocompatible material intended to maintain tooth vitality by acting as a protective barrier for the dental pulp. Similar to TheraCal LC, it is supplied in a syringe, eliminating the need for mixing, and is characterized by low solubility and a short setting time. In addition, its dual-curing capability represents a potential advantage over TheraCal LC, as it enables its use in procedures such as pulpotomy in primary molars. However, there is still limited evidence regarding its effectiveness and biocompatibility.[11]

NeoPUTTY™ is a new pre-mixed, bioactive, bioceramic, calcium silicate-based material. According to the manufacturer, this multipurpose MTA stimulates hydroxyapatite formation, promoting bioactivity and supporting tissue healing. NeoPUTTY is dimensionally stable with no shrinkage, providing a reliable seal and minimizing bacterial infiltration. Unlike conventional MTA, it exhibits immediate washout resistance, allowing the final restoration and crown cementation to be performed immediately after placement.[12] The putty-like material is supplied in a syringe with a plunger tip, enabling easy and rapid application while minimizing waste and reducing cost. Additionally, NeoPUTTY has no staining potential, since tantalum oxide is used for radiopacity instead of bismuth oxide, the primary cause of tooth discoloration associated with MTA. Being resin-free, the material may also have enhanced biocompatibility and bioactivity.[12]

In general, newly developed materials with modified composition should be thoroughly evaluated before clinical use. Given the lack of data on the interaction of NeoPUTTY with the pulpal tissue, and limited information available for TheraCal PT and TheraCal LC, this study aimed to investigate the in vivo biocompatibility of TheraCal PT, TheraCal LC and NeoPUTTY, following subcutaneous implantation in rats.

Material and methods

The pulp-capping materials used in the study were as follows: TheraCal PT (ThPT; BISCO, Schaumburg, USA); TheraCal LC (ThLC; BISCO) and NeoPUTTY (NPU; Avalon Biomed, Houston, USA). The composition of the materials is described in Table 1.

Study design and animals

According to the guidance and approval of the Institutional Animal Care and Use Committee of Cairo University (CU-IACUC), this experiment was conducted in the animal house of the Faculty of Medicine, Cairo University, Giza, Egypt, in accordance with the ARRIVE guidelines for in vivo animal research. Twenty adult male albino rats weighing 150–200 g were obtained from the animal house. The animals were housed in temperature-controlled rooms, and provided with food and water ad libitum. The care and maintenance of the experimental animals conformed to the International Guiding Principles for Biomedical Research Involving Animals. The rats were randomly divided into 2 groups (n = 10 per group) according to the sacrifice time points – 2 weeks and 4 weeks. These groups were further subdivided into 4 subgroups according to the material used (Table 2).

Sample size

According to a previous study,[13] a total sample of 20 rats (10 rats per group) was considered sufficient to detect an effect size of 1.23 with a statistical power of 0.80, using a two-sided hypothesis test and a significance level (α) of 0.05. The sample size was calculated using the G*power program (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower).[14]

Sample preparation

The 3 test materials were placed in sterile polyethylene tubes. TheraCal PT and TheraCal LC were light-cured for 20 s, using a light-curing unit (PenCure, J. Morita, Kyoto, Japan).

Anesthesia and the surgical protocol

The animals were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg of body weight (b.w.)) and xylazine hydrochloride (8 mg/kg b.w.). The back of each animal was shaved and disinfected with 10% betadine antiseptic solution (Mundipharma Egypt, Cairo, Egypt). Four incisions, 2 cm in length, were made on the back of each rat to create 4 pockets, into which polyethylene

Table 1. Pulp-capping materials used in the study

Commercial name	Manufacturer	Composition	Lot #
TheraCal PT	BISCO, Schaumburg, USA	synthetic Portland cement calcium silicate particles in a hydrophilic matrix	2000004394
TheraCal LC	BISCO, Schaumburg, USA	tricalcium silicate particles in a hydrophilic monomer	2000004742
NeoPUTTY	Avalon Biomed, Houston, USA	inorganic powder of tricalcium/dicalcium silicate in an organic medium	2020070101

Table 2. Study grouping

Groups	Sacrifice time	Subgroups	
Group 1 (n = 10)	2 weeks	MTA subgroup 1	polyethylene tubes filled with NeoPUTTY MTA (n = 10 tubes)
		TheraCal LC subgroup 1	polyethylene tubes filled with TheraCal LC (n = 10 tubes)
		TheraCal PT subgroup 1	polyethylene tubes filled with TheraCal PT (n = 10 tubes)
		control subgroup 1	empty polyethylene tubes (control) (n = 10 tubes)
Group 2 (n = 10)	4 weeks	MTA subgroup 2	polyethylene tubes filled with NeoPUTTY MTA (n = 10 tubes)
		TheraCal LC subgroup 2	polyethylene tubes filled with TheraCal LC (n = 10 tubes)
		TheraCal PT subgroup 2	polyethylene tubes filled with TheraCal PT (n = 10 tubes)
		control subgroup 2	empty polyethylene tubes (control) (n = 10 tubes)

MTA – mineral trioxide aggregate.

tubes were implanted. Three tubes were filled with the biomaterials under study, while the 4th was left empty as a negative control of the histological reaction. The tubes were placed with at least 2 cm distance between them to avoid interference in the tissue response. The skin was closed using 4/0 silk sutures (ISMC, Belbais, Egypt).

Postoperative care

Postoperatively, each animal received intramuscular injections of 10 mg/kg b.w. of amoxicillin and flucloxacillin (Flumox®; Eipico, 10th of Ramadan City, Egypt) to prevent secondary bacterial infection, and 10 mg/kg b.w. diclofenac potassium (Cataflam®; Novartis, Cairo, Egypt) for postoperative analgesia. In addition, a topical antibiotic spray containing neomycin (Bivatracin®; Egyptian Company for Advanced Pharmaceulticals, Cairo, Egypt) was applied to prevent local infection.

Animal sacrifice

The animals were euthanized by an intracardiac overdose of sodium thiopental (80 mg/kg b.w.) at 2 or 4 weeks after implantation, according to the experimental group.

Samples of skin and subcutaneous tissues containing the implants were excised with a safety margin of 1 cm. The tissues from one side of the tube interface were immediately fixed in 10% neutral buffered formalin for histopathological analysis. The tissues from the other side of the tube interface were frozen in liquid nitrogen and stored at –70°C for subsequent RNA extraction and gene expression analysis.[15]

Histopathological and histomorphometric analysis

The formalin-fixed tissue samples were dehydrated using ascending grades of alcohol, and then embedded in paraffin wax. The paraffin blocks were sectioned into 5-µm-thick slices and stained with hematoxylin and eosin (H&E) and Masson's trichrome (MT).

The H&E-stained sections were examined and photographed using a light microscope equipped with a digital camera (Leica, Heerbrugg, Switzerland) at magnifications of ×10, ×40 and ×100. The acquired images were analyzed using the ImageJ software, v. 1.53d (https://imagej.net/ij).[16] All quantitative analyses were performed on all animals (N = 20). For each animal, 3 non-serial sections were examined for each material, and the mean value was calculated. Morphometric analyses were conducted by a single calibrated and blinded examiner.

Number and density of inflammatory cells

The number of inflammatory cells was estimated within the connective tissue capsules. For the detection of inflammatory cell number and density, The H&E-stained sections were examined using a light microscope equipped with a digital camera (Leica) at ×40 magnification. An image of a standardized field of the capsule in close proximity to the tube opening was captured.[17] The acquired images were analyzed using the ImageJ software, v. 1.53d (https://imagej.net/ij).[16] For each field, the total number of inflammatory cells, including neutrophils, lymphocytes, plasma cells, macrophages, and giant cells, was determined. The density of inflammatory cells (the number of cells per square millimeter of the capsule) was then calculated by dividing the total number of inflammatory cells by the total area of the capsule field, as previously described.[17–19]

Grade of connective tissue capsule inflammation

According to the number of inflammatory cells, the inflammatory reaction was graded as follows: Grade 0 – no inflammatory cells detected; Grade 1 – fewer than 25 cells detected; Grade 2 – 25–125 cells detected; and Grade 3 – more than 125 cells detected.[20,21]

Grade of inflammation extension

The histological images were also graded according to the extent of inflammatory cell spread within the connective tissue capsule and the surrounding connective tissue as follows: Grade 0 – no inflammatory cells detected; Grade 1 – inflammatory cells confined to the area of the capsule adjacent to the implanted tubes; Grade 2 – inflammatory cells restricted to the capsule without extension into the surrounding connective tissue; and Grade 3 – inflammatory cells extending beyond the capsule into the surrounding connective tissue.[22]

Capsule thickness

For the evaluation of capsular thickness, the H&E-stained sections were examined using a light microscope equipped with a digital camera (Leica) at ×10 magnification. The acquired images were analyzed using the ImageJ software, v. 1.53d (https://imagej.net/ij).[16] The thickness of the central part of the capsule was measured in 3 non-serial sections, and the mean value was calculated for each material.[19]

Area percentage of collagen fibers within the capsule

The MT-stained sections were examined using a light microscope equipped with a digital camera (Leica) at ×40 magnification. The acquired images were analyzed using the ImageJ software, v. 1.53d (https://imagej.net/ij).[16] The area percentage of collagen fibers within the capsule was measured in the MT-stained sections using ImageJ, as previously described.[23]

Quantitative real-time polymerase chain reaction (qRT-PCR) assays

Quantitative real-time polymerase chain reaction (qRT-PCR) assays were performed using a real-time thermal detection system with total RNA, the iScript™ one-step RT-PCR kit with SYBR® Green (Bio-Rad Laboratories, Hercules, USA), and specific primers. The gene-specific primer sequences used for amplification are listed in Table 3. β-actin was used as the internal control. Relative gene expression levels of vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6) were quantified using the $2^{-\Delta\Delta CT}$ method.

Table 3. Primer sequences of all studied genes

Gene	Primer sequence from 5' to 3'
VEGF	forward: 5'-CACGACAGAAGGGGAGCAGAA-3'
	reverse: 5'-TGCGGATCTTGGACAAACAAAT-3'
IL-6	forward: 5'-TCCTACCCCAACTTCCAATGCTC-3'
	reverse: 5'-TTGGATGGTCTTGGTCCTTAGCC-3'
β-actin	forward: 5'-GTGACATCCACACCCAGAGG-3'
	reverse: 5'-ACAGGATGTCAAAACTGCCC-3'

Statistical analysis

Data obtained from both the histomorphometric analysis and the RT-PCR assays, estimated at 2 and 4 weeks, was expressed as mean ± standard deviation ($M \pm SD$). Normally distributed parametric data was analyzed using one-way or two-way analysis of variance (ANOVA) tests. Tukey's post hoc test was applied for multiple pairwise comparisons when the ANOVA results were statistically significant. Non-parametric data was analyzed using the Kruskal–Wallis test followed by Dunn's post hoc test for statistically significant results. A p-value <0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS), v. 15.0 (SPSS Inc., Chicago, USA).

Results

Histopathological results

Group 1 (two-week interval)

H&E staining:

MTA subgroup 1 (Fig. 1A, 1B) and TheraCal LC subgroup 1 (Fig. 1C, 1D) revealed a thick connective tissue capsule with a moderate inflammatory reaction. The aggregations of chronic inflammatory cells were observed within the unorganized fibrous connective tissue containing numerous plasma cells and lymphocytes, along with some fibroblasts. Mineralization was observed in both groups (Fig. 1A, 1C). At higher magnification, congested blood vessels were evident in the MTA subgroup 1 (Fig. 1B), while multinucleated giant cells were observed in the TheraCal LC subgroup 1 (Fig. 1D). TheraCal PT subgroup 1 exhibited a mild inflammatory reaction with a thinner capsule. The remnants of the implanted material were also observed (Fig. 1E, 1F). The control subgroup 1 showed more organized connective tissue with a mild inflammatory reaction and few lymphocytes (Fig. 1G, 1H). Fibroblasts were observed in all subgroups of group 1.

MT staining:

MTA subgroup 1 (Fig. 2A) and TheraCal LC subgroup 1 (Fig. 2B) revealed slightly stained, thin and irregularly arranged collagen fibers within the inflammatory capsule. TheraCal PT subgroup 1 showed prominent deposition of thin, irregular collagen fibers (Fig. 2C). The control sub-

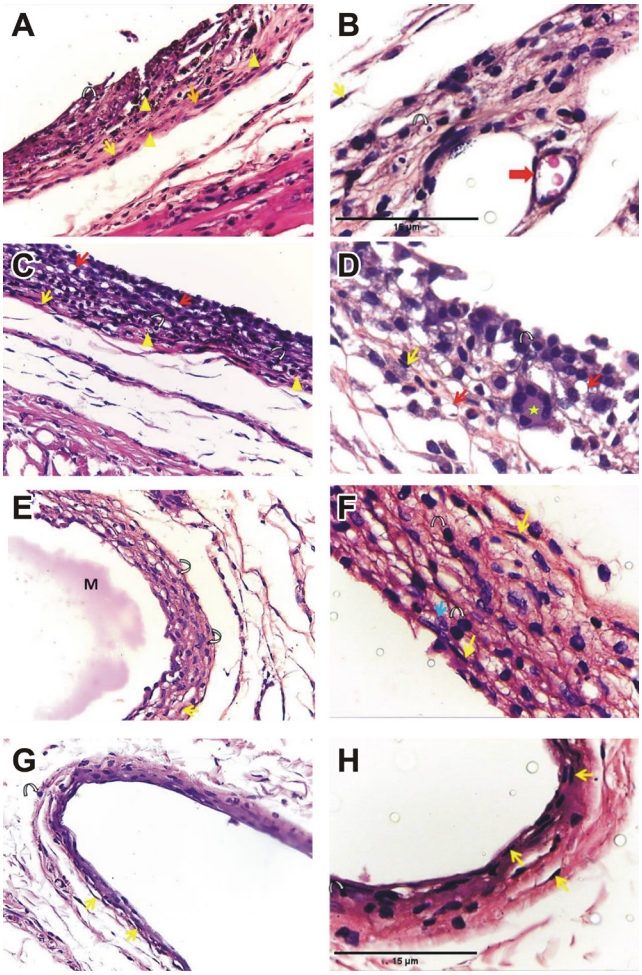

Fig. 1. Histopathological examination of the subcutaneous connective tissue responses to all the tested pulp-capping materials and the empty tube (control) in rats at 2 weeks (group 1); hematoxylin and eosin (H&E) staining

A, B – MTA subgroup 1 (A – moderate inflammatory reaction, the tissue packed with inflammatory cells, with multiple areas of mineralization (triangle), B – congested blood vessels (red arrow)); C, D – TheraCal LC subgroup 1 (C – moderate inflammatory reaction, the tissue packed with inflammatory cells, with few areas of mineralization (triangle), D – multinucleated giant cells (yellow star)); E, F – TheraCal PT subgroup 1 (E – mild inflammatory reaction, remnants of the material (M), F – some inflammatory cells); G, H – control subgroup 1 (mild inflammatory reaction, inflammatory cells). Cells observed: white arrow – lymphocytes; yellow arrow – fibroblasts; blue arrow – plasma cells.
A, C, E, G ×40 magnification; B, D, F, H ×100 magnification.

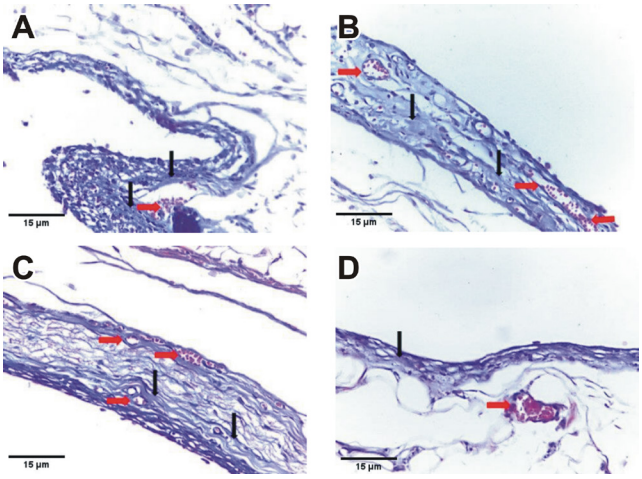

Fig. 2. Histopathological examination of the subcutaneous connective tissue responses to all the tested pulp-capping materials and the empty tube (control) in rats at 2 weeks (group 1); Masson's trichrome (MT) staining

A – MTA subgroup 1; B – TheraCal LC subgroup 1; C – TheraCal PT subgroup 1; D – control subgroup 1; slightly stained collagen in a meshwork arrangement (black arrow), associated with congested vessels (red arrow) in all subgroups (A–D).
×40 magnification.

group 1 showed mild collagen fiber deposition (faint blue) with fibroblasts and a few capillaries (Fig. 2D). Congested blood vessels were observed in all subgroups of group 1.

Group 2 (four-week interval)

H&E staining:

MTA subgroup 2 revealed a relatively thick fibrous capsule with limited organization and mild peri-capsular fibrosis, along with some congested capillaries (Fig. 3A, 3B). TheraCal LC subgroup 2 showed disorganized reactionary tissue with edema between collagen fibers and scattered inflammatory cell infiltration (Fig. 3C, 3D). TheraCal PT subgroup 2 demonstrated a relatively organized fibrous capsule with several active fibroblasts characterized by swollen, large nuclei and abundant cytoplasm. A marked decrease in inflammatory cell infiltration with mild peri-capsular fibrosis was also observed (Fig. 3E, 3F). The control subgroup 2 exhibited uniform collagen fiber formation. Collagen fibers were thin and discrete, with mild inflammatory infiltrate, mainly represented by lymphocytes and interspersed fibroblasts (Fig. 3G, 3H).

MT staining:

MTA subgroup 2 (Fig. 4A) and TheraCal LC subgroup 2 (Fig. 4B) revealed several fibroblasts within a band of dense collagen fibers, which stained lighter with trichrome as compared to the collagen fibers observed in mature granulation tissue. Fibroblasts and blood vessels were also present, and inflammatory cells, mainly lymphocytes, were evident. TheraCal PT subgroup 2 (Fig. 4C) and the control subgroup 2 (Fig. 4D) showed deposition of regularly arranged collagen fibers, along with evidence of newly formed blood vessels.

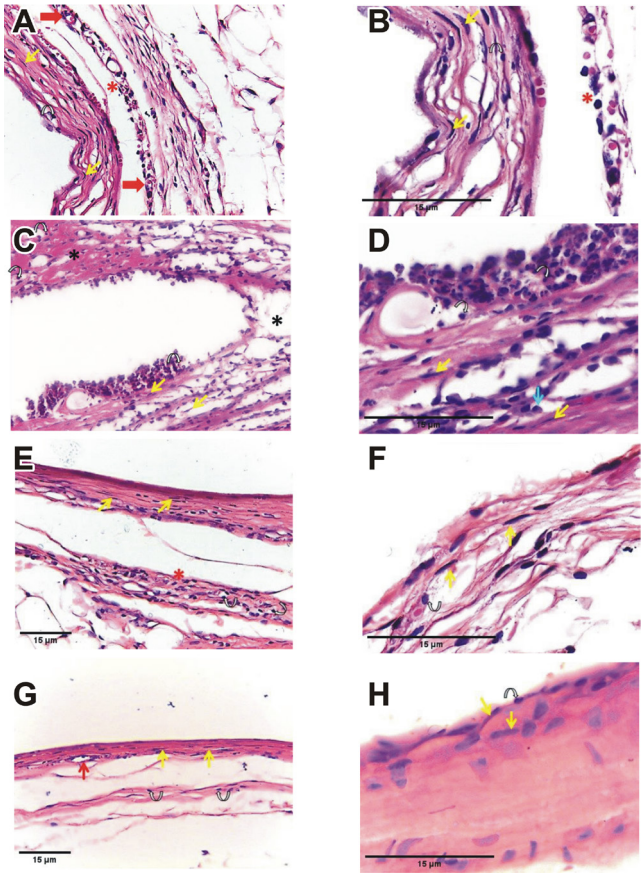

Fig. 3. Histopathological examination of the subcutaneous connective tissue responses to all the tested pulp-capping materials and the empty tube (control) in rats at 4 weeks (group 2); hematoxylin and eosin (H&E) staining

A, B – MTA subgroup 2 (A – mild inflammatory reaction with some inflammatory cells and congested capillaries (red arrow), B – mild peri-capsular fibrosis (red asterisk)); C, D – TheraCal LC subgroup 2 (C – disorganized reactionary tissue with edema (black asterisk), D – chronic inflammatory cell infiltrate); E, F – TheraCal PT subgroup 2 (E – well-organized reactionary tissue with dense collagen fibers, occasionally infiltrated by lymphocytes, F – active fibroblasts); G, H – control subgroup 2 (thin reactionary tissue with organized collagen fibers, fibroblasts and a mild inflammatory reaction). Cells observed: white arrow – lymphocytes; yellow arrow – fibroblasts; blue arrow – plasma cells.
A, C, E, G ×40 magnification; B, D, F, H ×100 magnification.

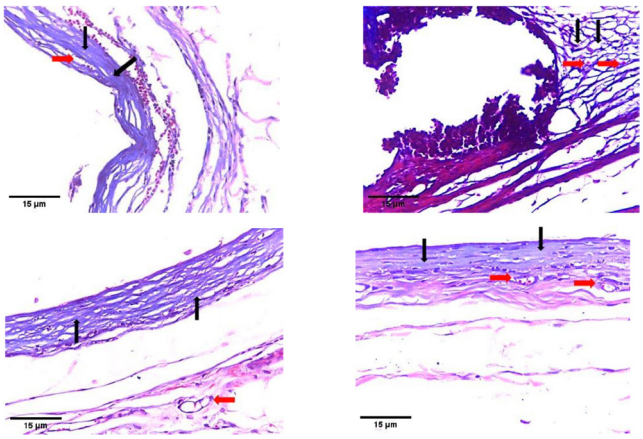

Fig. 4. Histopathological examination of the subcutaneous connective tissue responses to all the tested pulp-capping materials and the empty tube (control) in rats at 4 weeks (group 2); Masson's trichrome (MT) staining

A – MTA subgroup 2; B – TheraCal LC subgroup 2; C – TheraCal PT subgroup 2; D – control subgroup 2; collagen fibers forming a mesh (black arrow) amongst inflammatory cells, blood vessels (red arrow) in all subgroups (A–D).
×40 magnification.

Number and density of inflammatory cells

Statistically significantly lower mean values for both the number and density of inflammatory cells were recorded in the control subgroup, followed by the TheraCal PT subgroup, as compared to the TheraCal LC and MTA subgroups at both observational time points (group 1 and group 2). No significant difference was found between the TheraCal LC and MTA subgroups at either time point. A statistically significant time-dependent decrease in the number and density of inflammatory cells was observed in the TheraCal PT, TheraCal LC and MTA subgroups (Table 4, Fig. 5A, 5B, Supplementary Table 1).

Capsule thickness

A statistically significantly lower mean value of the capsule thickness was recorded in the control subgroup, followed by the TheraCal PT subgroup, as compared to the TheraCal LC and MTA subgroups at both observational time points (group 1 and group 2). No significant difference was found between the TheraCal LC and MTA subgroups at either time point. A statistically significant time-dependent increase in the capsule thickness was observed in all subgroups (Table 4, Fig. 5C, Supplementary Table 1).

Table 4. Results for the number and density of inflammatory cells, the capsule thickness, and the area percentage of collagen fibers within the capsule

Parameter	Group	Subgroup	*M* ±*SD*	*SE*	*F*	*p*-value
Number of inflammatory cells (*n*)	group 1	MTA subgroup 1	90.4 ±29.7^{A}	9.39	7.27	0.000*
		TheraCal LC subgroup 1	92.0 ±33.8^{A}	10.70		
		TheraCal PT subgroup 1	46.5 ±14.2^{B}	4.49		
		control subgroup 1	18.8 ±6.6^{CD}	2.07		
	group 2	MTA subgroup 2	36.1 ±1.7^{BC}	0.53		
		TheraCal LC subgroup 2	38.4 ±7.2^{BC}	2.26		
		TheraCal PT subgroup 2	12.0 ±3.2^{D}	1.01		
		control subgroup 2	8.3 ±2.5^{D}	0.80		
Density of inflammatory cells [*n*/mm^2]	group 1	MTA subgroup 1	0.973 ±0.320^{A}	0.101	7.27	0.000*
		TheraCal LC subgroup 1	0.991 ±0.364^{A}	0.115		
		TheraCal PT subgroup 1	0.501 ±0.153^{B}	0.048		
		control subgroup 1	0.202 ±0.071^{CD}	0.022		
	group 2	MTA subgroup 2	0.388 ±0.018^{BC}	0.006		
		TheraCal LC subgroup 2	0.414 ±0.077^{BC}	0.024		
		TheraCal PT subgroup 2	0.129 ±0.034^{D}	0.011		
		control subgroup 2	0.089 ±0.027^{D}	0.009		
Capsule thickness [mm]	group 1	MTA subgroup 1	20.68 ±6.31^{BC}	2.00	4.09	0.010*
		TheraCal LC subgroup 1	26.03 ±4.19^{B}	1.32		
		TheraCal PT subgroup 1	11.43 ±2.60^{D}	0.82		
		control subgroup 1	5.20 ±1.27^{E}	0.40		
	group 2	MTA subgroup 2	38.06 ±6.00^{A}	1.90		
		TheraCal LC subgroup 2	42.51 ±5.40^{A}	1.71		
		TheraCal PT subgroup 2	21.59 ±1.80^{BC}	0.57		
		control subgroup 2	15.89 ±2.34^{CD}	0.74		
Area percentage of collagen fibers within the capsule [%]	group 1	MTA subgroup 1	55.85 ±3.76^{AB}	1.19	16.75	0.000*
		TheraCal LC subgroup 1	63.06 ±7.26^{A}	2.30		
		TheraCal PT subgroup 1	50.20 ±9.43^{BC}	2.98		
		control subgroup 1	42.32 ±1.68^{C}	1.68		
	group 2	MTA subgroup 2	68.66 ±4.79^{A}	1.52	26.03	0.000*
		TheraCal LC subgroup 2	73.05 ±5.87^{A}	1.86		
		TheraCal PT subgroup 2	60.74 ±9.27^{B}	2.93		
		control subgroup 2	50.70 ±2.18^{C}	0.69		

M – mean; *SD* – standard deviation; *SE* – standard error; * statistically significant; means with different superscript letters are significantly different.

Area percentage of collagen fibers within the capsule (MT stain)

Two-way ANOVA revealed no significant interaction between treatment and time, while a significant difference was detected among the different kinds of treatment within each time period. Therefore, one-way ANOVA was used to compare the subgroups at each time point.

One-way ANOVA for the 2-week subgroups showed a statistically significantly lower mean value in the control subgroup 1, followed by the TheraCal PT subgroup 1, as compared to the TheraCal LC and MTA subgroups 1. Similarly, one-way ANOVA for the 4-week subgroups demonstrated a statistically significantly lower mean value in the control subgroup 2, followed by the TheraCal PT subgroup 2. No statistically significant difference was found between the TheraCal LC and MTA subgroups at either observational time point (group 1 and group 2) (Table 4, Fig. 6, Supplementary Table 1).

Grade of connective tissue capsule inflammation and the grade of inflammation spread into the adjacent connective tissue

Regarding group 1 (2-week time point), most specimens in the control subgroup 1 exhibited Grade 1 connective tissue capsule inflammation, whereas the majority of specimens in the TheraCal PT, TheraCal LC and MTA subgroups 1 showed Grade 2 inflammation. A statistically significantly lower value was observed in the control subgroup 1 as compared to the other groups, while no statistically significant difference was found among the TheraCal PT, TheraCal LC and MTA subgroups 1. At the 4-week time point (group 2), all specimens in the control subgroup 2 and the TheraCal PT subgroup 2 exhibited Grade 1 inflammation. The majority of specimens in the TheraCal LC subgroup 2 showed Grade 2 inflammation, whereas half of the specimens in the MTA subgroup 2 exhibited Grade 1 inflammation and the other half showed

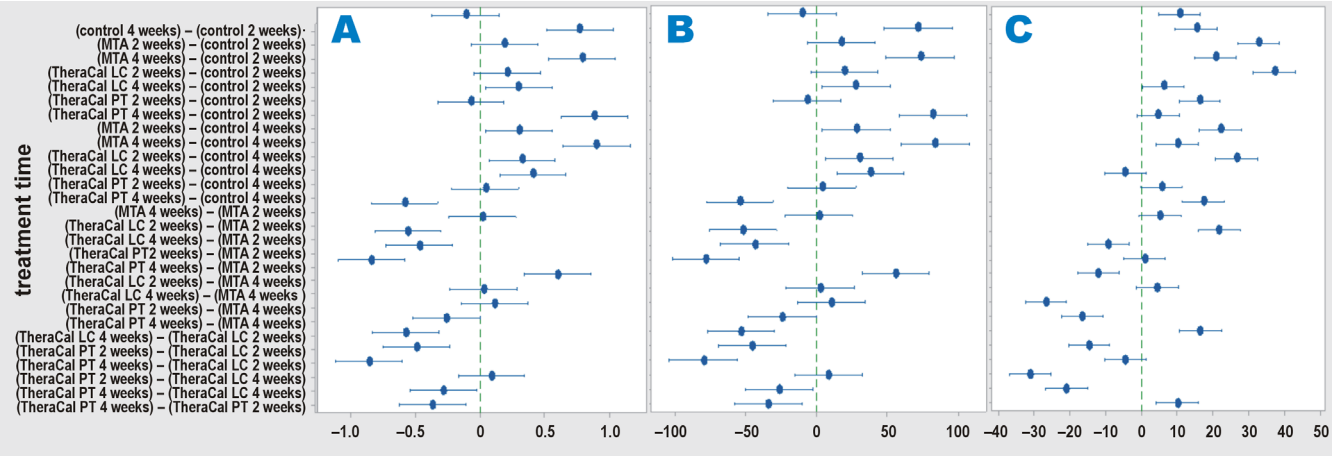

Fig. 5. Tukey's post hoc test results

A – density of inflammatory cells; B – number of inflammatory cells; C – capsule thickness.
95% confidence interval (*CI*). If an interval does not contain zero, the corresponding means are significantly different.

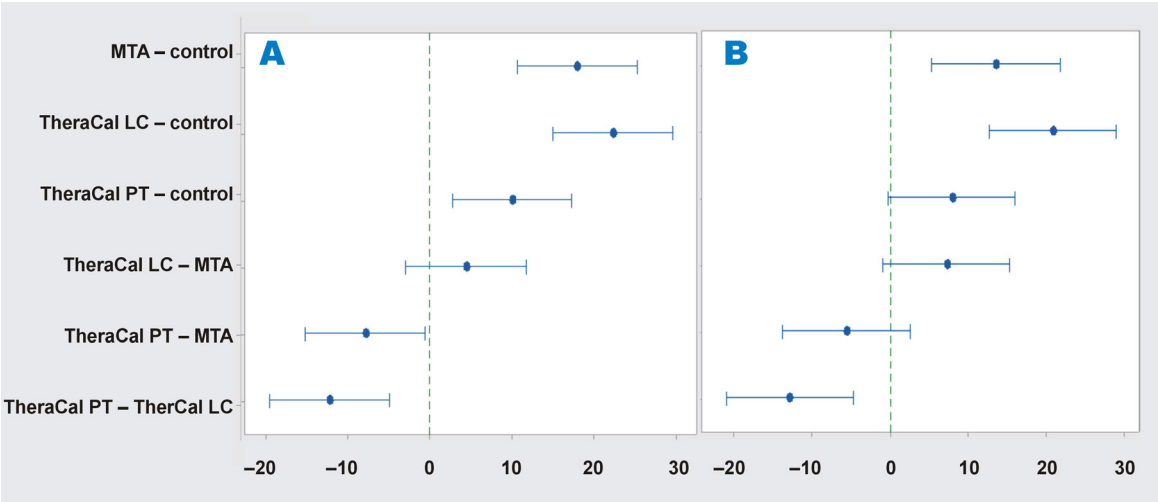

Fig. 6. Tukey's post hoc test results for the area percentage of collagen fibers within the capsule

A – at 2 weeks; B – at 4 weeks.
95% *CI*. If an interval does not contain zero, the corresponding means are significantly different.

Table 5. Kruskal–Wallis test results for the grade of connective tissue capsule inflammation and the grade of inflammation spread into the adjacent connective tissue

Parameter	Group	Subgroup	Grade (*n*)			*p*-value
			G1	G2	G3	
Grade of connective tissue capsule inflammation	group 1	MTA subgroup 1[A]	0	9	1	0.000*
		TheraCal LC subgroup 1[A]	0	8	2	
		TheraCal PT subgroup 1[A]	0	10	0	
		control subgroup 1[B]	8	2	0	
	group 2	MTA subgroup 2[A]	5	5	0	0.001*
		TheraCal LC subgroup 2[A]	3	7	0	
		TheraCal PT subgroup 2[B]	10	0	0	
		control subgroup 2[B]	10	0	0	
Grade of inflammation spread into the adjacent connective tissue	group 1	MTA subgroup 1[A]	4	1	5	0.000*
		TherCal LC subgroup 1[A]	1	0	9	
		TherCal PT subgroup 1[B]	8	2	0	
		control subgroup 1[B]	8	2	0	
	group 2	MTA subgroup 2[A]	0	5	5	0.000*
		TherCal LC subgroup 2[A]	0	3	7	
		TherCal PT subgroup 2[B]	8	1	1	
		control subgroup 2[B]	9	0	1	

* statistically significant; means with different superscript letters are significantly different.

Grade 2 inflammation. The differences between the TheraCal PT subgroup 2 and both the TheraCal LC subgroup 2 and the MTA subgroup 2 were statistically significant, while the difference between the MTA and TheraCal LC subgroups 2 was not statistically significant (Table 5, Supplementary Table 2).

At both observational time points, most specimens in the control and TheraCal PT subgroups exhibited Grade 1 inflammation spread into the adjacent connective tissue, whereas most specimens in the TheraCal LC and MTA subgroups exhibited Grade 3. The differences between the TheraCal PT subgroup and both the TheraCal LC and MTA subgroups were statistically significant, while the difference between the MTA and TheraCal LC subgroups was not statistically significant at either observational time point (group 1 and group 2) (Table 5, Supplementary Table 2).

A statistically significant decrease in both the grade of inflammation and the grade of inflammation spread into the adjacent connective tissue was observed over time in all subgroups (Table 5, Supplementary Table 2).

Quantitative RT-PCR analysis

Regarding the VEGF mRNA gene expression levels, statistical analysis demonstrated a statistically significantly lower mean value in the TheraCal PT subgroup as compared to the TheraCal LC and MTA subgroups at both observational time points. A statistically significant lower value was also detected in the MTA subgroup as compared to the TheraCal LC subgroup. A statistically significant decrease in VEGF gene expression over time was observed in the TheraCal PT and TheraCal LC subgroups (Table 6, Fig. 7, Supplementary Table 3).

Statistical analysis of mRNA gene expression of IL-6 revealed a statistically significantly lower mean value in the TheraCal PT subgroup as compared to both the TheraCal LC and MTA subgroups, while no significant difference was detected between the TheraCal LC and MTA subgroups at either observational time point. A statistically significant decrease in IL-6 gene expression was observed for all 3 materials over time (Table 6, Fig. 7, Supplementary Table 3).

Discussion

Vital pulp therapy is a promising individualized treatment approach for managing irreversible pulpitis and promoting dentin bridge formation. Biocompatibility is an essential characteristic that must be considered in the case of the materials applied directly to the exposed pulp tissue.[24] Materials intended for direct pulp capping should be non-cytotoxic to dental pulp stem cells and bioactive in promoting odontogenic differentiation. However, in vitro cell culture assays cannot fully predict in vivo tissue responses. Therefore, in addition to cytotoxicity tests, animal studies and clinical evaluations are necessary to safeguard against the potential adverse effects of newly introduced dental materials.[25]

Subcutaneous implantation in vivo is considered one of the most reliable methods for assessing the

Table 6. Two-way ANOVA results for vascular endothelial growth factor (VEGF) and interleukin-6 (IL-6) gene expression

Gene expression	Group	Subgroup	*M* ±*SD*	*SE*	*F*	*p*-value
VEGF	group 1[A]	MTA subgroup 1	0.3757 ±0.0723[D]	0.0229	4.33	0.007*
		TheraCal LC subgroup 1	0.6205 ±0.0515[B]	0.0107		
		TheraCal PT subgroup 1	0.3015 ±0.0339[E]	0.0163		
		control subgroup 1	1[A]	0		
	group 2[B]	MTA subgroup 2	0.3492 ±0.0282[DE]	0.0089		
		TheraCal LC subgroup 2	0.5475 ±0.0309[C]	0.0124		
		TherCal PT subgroup 2	0.2271 ±0.0394[F]	0.0098		
		control subgroup 2	1[A]	0		
IL-6	group 1[A]	MTA subgroup 1	0.37 ±0.01[B]	0.003	15.43	0.000*
		TheraCal LC subgroup 1	0.38 ±0.05[B]	0.015		
		TheraCal PT subgroup 1	0.29 ±0.03[C]	0.008		
		control subgroup 1	1[A]	0		
	group 2[B]	MTA subgroup 2	0.27 ±0.03[C]	0.010		
		TheraCal LC subgroup 2	0.29 ±0.03[C]	0.009		
		TherCal PT subgroup 2	0.22 ±0.02[D]	0.007		
		control subgroup 2	1[A]	0		

* statistically significant; means with different superscript letters are significantly different.

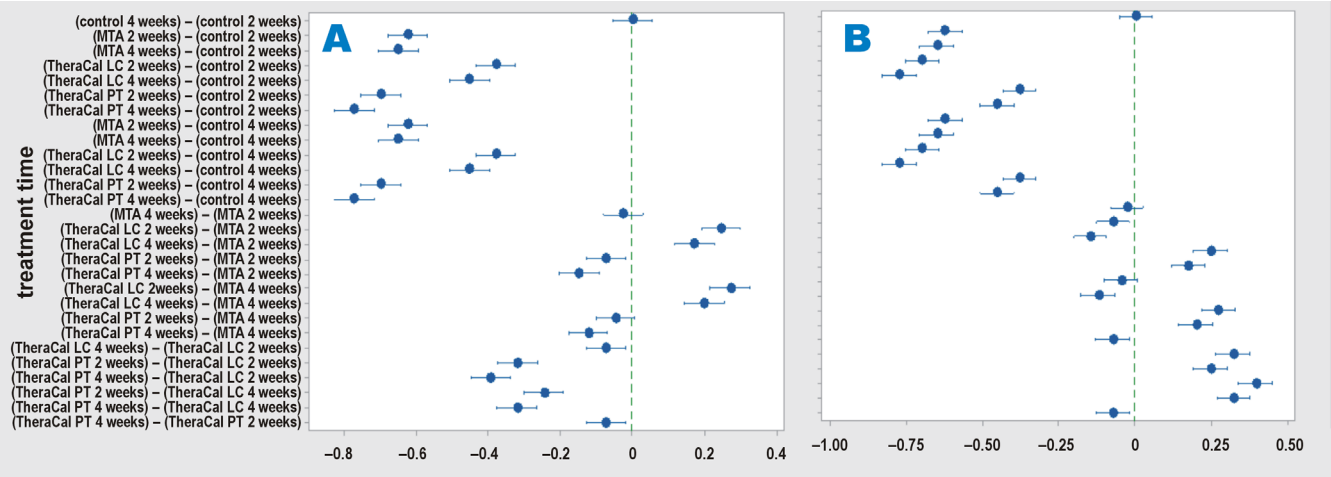

Fig. 7. Dot plot showing Tukey's post hoc test results for gene expression

A – vascular endothelial growth factor (VEGF); B – interleukin-6 (IL-6).
95% *CI*. If an interval does not contain zero, the corresponding means are significantly different.

biocompatibility of dental materials.[26,27] Based on this concept, the present study aimed to evaluate the biological characteristics of MTA (NeoPUTTY), Theracal LC and Theracal PT, following subcutaneous implantation in rats.

The pulp-capping material extruded from the opening of the polyethylene tube comes into direct contact with the surrounding tissues, triggering an inflammatory response similar to that observed in vital pulp therapy. Inert polyethylene tubes were used for implantation, since they allow the tested material to remain in close apposition to the tissues.[26] An additional empty tube was implanted as a control to standardize variables, minimize selection bias, and neutralize the potential confounding factors that could affect the results.[28] The follow-up periods were set at 2 and 4 weeks, following Silva-Herzog et al., who reported that 14 days are sufficient to evaluate the initial phase of material biocompatibility.[29]

At 2 weeks, the inflammatory responses observed in the control subgroup 1 and in all the evaluated materials (NeoPUTTY, TheraCal LC and TheraCal PT) were more pronounced than those observed at 4 weeks. In addition to the potential toxic effects of the implanted materials and the tube material, the surgical trauma induced during tube insertion might also contribute to this response.[30] Giraud et al. suggested that there exists a dynamic equilibrium between inflammation and regeneration, and

that this balance may shift toward either regeneration or inflammation, depending on the restorative material used.[31]

Moderate inflammation was the characteristic tissue response to MTA, which decreased after 4 weeks of exposure to the material. These findings are consistent with those of Shahi et al., who reported the presence of collagen fibers, fibrosis and persistent inflammatory cell infiltration after 21 days.[32] Inflammation may result from multiple factors; however, the initial inflammatory response can be triggered by factors such as cytokine production (e.g., IL-1 and IL-6), as well as the high pH and setting temperature of the material.[33] The formation of calcium deposits in the subcutaneous connective tissue indicates the osteoinductive potential of the tested material.[34] The mineralization observed in the MTA subgroup may be attributed to the reaction between calcium ions from calcium hydroxide and carbon dioxide present in the connective tissue. Calcium hydroxide is formed from calcium phosphate and calcium oxide released during the setting reaction of MTA.[32]

The histological examination and histomorphometric analysis of the TheraCal LC subgroup 1 demonstrated the presence of a thick connective tissue capsule with moderate aggregations of chronic inflammatory cells. The resin component of TheraCal LC may lead to the release of residual monomers, which can diffuse into the surrounding tissues, exert cytotoxic effects and stimulate the production of pro-inflammatory cytokines.[31] It has been suggested that TheraCal LC may shift the biological balance toward inflammation, whereas resin-free materials tend to exhibit anti-inflammatory properties and promote tissue regeneration.[24] Similarly, the histological examination of TheraCal LC used in primary teeth with deep carious lesions and symptoms of reversible pulpitis revealed the disruption of the odontoblastic layer, fibrosis and dystrophic calcification, along with varying degrees of pulpitis in the examined specimens, indicating that none of the samples presented completely healthy pulp tissue.[35]

In addition to Portland cement powder, TheraCal LC contains a hydrophilic resin monomer, a hydrophobic resin monomer and a hydrophilic fillers.[36] Methacrylate resin components can adversely affect cell membranes by disrupting the lipid bilayer. Previous studies have demonstrated that the exposure of human dental pulp stem cells to TheraCal LC results in impaired cellular metabolism and the formation of inadequate tissue bridges.[37,38] Furthermore, the cells cultured in the media containing TheraCal LC exhibit markedly elevated levels of IL-8.[39] Evidence of mineralization was observed in the TheraCal LC subgroup, which is consistent with the findings of Hinata et al., who evaluated the ability of TheraCal LC to form apatite-like precipitates at 7, 14, and 28 days after subcutaneous implantation in rats.[40] Although TheraCal LC has been reported not to produce calcium hydroxide during hydration, calcium ion release and calcium phosphate deposition have been detected on its surface.[36] This phenomenon may be attributed to the resin-modified nature of TheraCal LC, which limits ion exchange with the surrounding environment. Consequently, the water absorption and calcium ion release associated with cement hydration may be restricted due to limited ion transport across and within the resin matrix.

The TheraCal PT subgroup demonstrated a mild inflammatory response that decreased over time, along with a lower percentage area of collagen fibers within the fibrous capsule as compared to the MTA and TheraCal LC subgroups. These findings suggest that TheraCal PT exhibits a lower cytotoxic effect on the surrounding tissues. The present results are consistent with those reported by Rodríguez-Lozano et al., who found that the number of viable cells in the TheraCal LC-treated group was lower than in the TheraCal PT-treated group, as determined by an apoptosis/necrosis assay.[41] The cytotoxicity of TheraCal LC has been hypothesized to result from the unpolymerized resin monomers that remain after polymerization.[42,43] Additionally, some studies have shown that cured TheraCal LC releases specific additives, such as ethyl 4-(dimethylamino)benzoate and camphorquinone.[44] The primary human pulp fibroblasts exposed to camphorquinone have demonstrated an increase in the production of reactive oxygen species (ROS).[45,46] This evidence suggests that the light-curing components released from TheraCal LC may contribute to cell death by enhancing the generation of ROS. The absence of mineralization observed in the TheraCal PT group could be attributed to its composition. According to the manufacturer's datasheet, TheraCal PT is a resin-modified calcium silicate cement composed of poly(ethylene glycol) dimethacrylate (PEGDMA) (10–30%) and bisphenol A-glycidyl methacrylate (Bis-GMA) (5–10%). However, the datasheet does not explicitly mention the presence of calcium in its formulation.[47]

Pulpal fibroblasts have been shown to play a significant role in the initiation of inflammatory responses.[48] In addition, pro-inflammatory cytokines such as VEGF and IL-6, as well as complement fragments C3a and C5a, have been reported to promote the recruitment and activation of cells within an inflammatory microenvironment.[49,50] Consistent with these findings, the results of the present study demonstrated that MTA and TheraCal LC induced higher levels of VEGF and IL-6 release as compared to the TheraCal PT and control groups. Similarly, TheraCal LC has been reported to increase the secretion of VEGF and IL-6 from pulpal fibroblasts in vitro.[51]

Although numerous studies have investigated the biological characteristics and biocompatibility of calcium silicate-based cements, including those evaluated in the present study, the rapid introduction of new materials to the market necessitates the continuous and updated biological as well as chemical-mechanical profiling of each formulation prior to clinical application. This highlights

the importance and relevance of animal studies as an initial approach for evaluating novel materials. However, these findings should be interpreted with caution, as the performance of the tested materials may be influenced by several extrinsic factors under clinical conditions.

Conclusions

The findings of the present study demonstrated that TheraCal PT exhibited superior biocompatibility in vivo in comparison with NeoPUTTY (MTA) and TheraCal LC. Therefore, TheraCal PT may be considered a promising material for direct pulp capping. Nevertheless, further studies are recommended to evaluate the additional properties of these materials before their widespread clinical application.

Ethics approval and consent to participate

According to the guidance and approval of the Institutional Animal Care and Use Committee of Cairo University (CU-IACUC), this experiment was conducted in the animal house of the Faculty of Medicine, Cairo University, Giza, Egypt, in accordance with the ARRIVE guidelines for in vivo animal research.

Data availability

The datasets supporting the findings of the current study, including the supplementary material, are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Shitie A, Addis R, Tilahun A, Negash W. Prevalence of dental caries and its associated factors among primary school children in Ethiopia. *Int J Dent*. 2021;2021:6637196. doi:10.1155/2021/6637196
2. Karimi M. Importance of preserving deciduous teeth in childhood. *SAODS*. 2020;3(12):1–5. https://saods.net/wp-content/uploads/SAODS/PDF-Data/2020/Volume-3-Issue-12/SAODS-03-0189.pdf. Accessed January 1, 2023.
3. Kratunova E, Silva D. Pulp therapy for primary and immature permanent teeth: An overview. *Gen Dent*. 2018;66(6):30–38. PMID:30444704.
4. Al Tuwirqi AA, El Ashiry EA, Alzahrani AY, Bamashmous N, Bakhsh TA. Tomographic evaluation of the internal adaptation for recent calcium silicate-based pulp capping materials in primary teeth. *Biomed Res Int*. 2021;2021:5523145. doi:10.1155/2021/5523145
5. Rajasekharan S, Martens LC, Vandenbulcke J, Jacquet W, Bottenberg P, Cauwels RG. Efficacy of three different pulpotomy agents in primary molars: A randomized control trial. *Int Endod J*. 2017;50(3):215–228. doi:10.1111/iej.12619
6. Kim JR, Nosrat A, Fouad AF. Interfacial characteristics of Biodentine and MTA with dentine in simulated body fluid. *J Dent*. 2015;43(2):241–247. doi:10.1016/j.jdent.2014.11.004
7. Parirokh M, Torabinejad M. Mineral trioxide aggregate: A comprehensive literature review – Part III: Clinical applications, drawbacks, and mechanism of action. *J Endod*. 2010;36(3):400–413. doi:10.1016/j.joen.2009.09.009
8. Cannon M, Gerodias N, Viera A, Percinoto C, Jurado R. Primate pulpal healing after exposure and TheraCal application. *J Clin Pediatr Dent*. 2014;38(4):333–337. doi:10.17796/jcpd.38.4.m585322121536q71
9. Gandolfi MG, Siboni F, Botero T, Bossù M, Riccitiello F, Prati C. Calcium silicate and calcium hydroxide materials for pulp capping: Biointeractivity, porosity, solubility and bioactivity of current formulations. *J Appl Biomater Funct Mater*. 2015;13(1):43–60. doi:10.5301/jabfm.5000201
10. Arandi NZ, Rabi T. TheraCal LC: From biochemical and bioactive properties to clinical applications. *Int J Dent*. 2018;2018:3484653. doi:10.1155/2018/3484653
11. Sanz JL, Soler-Doria A, López-García S, et al. Comparative biological properties and mineralization potential of 3 endodontic materials for vital pulp therapy: Theracal PT, Theracal LC, and Biodentine on human dental pulp stem cells. *J Endod*. 2021;47(12):1896–1906. doi:10.1016/j.joen.2021.08.001
12. Marciano MA, Hungaro Duarte MA, Camilleri J. Dental discoloration caused by bismuth oxide in MTA in the presence of sodium hypochlorite. *Clin Oral Investig*. 2015;19(9):2201–2209. doi:10.1007/s00784-015-1466-8
13. Sak Mortaş E. Evaluation of the biocompatibility of a light-cured pulp-coating material in the subcutaneous tissue of rats [in Turkish]. Specialization thesis. Karadeniz Technical University, Faculty of Dentistry, Department of Restorative Dentistry; 2016. https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=VeoDA9hGcEmVtMn8GRhcFA&no=nzTcFTYBiZaOOnpDhcymLw. Accessed April 1, 2022.
14. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods*. 2007;39(2):175–191. doi:10.3758/bf03193146
15. Hulsart-Billström G, Stelzl C, Procter P, et al. In vivo safety assessment of a bio-inspired bone adhesive. *J Mater Sci Mater Med*. 2020;31(2):24. doi:10.1007/s10856-020-6362-3
16. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods*. 2012;9(7):671–675. doi:10.1038/nmeth.2089
17. Da Fonseca TS, Da Silva GF, Tanomaru-Filho M, Sasso-Cerri E, Guerreiro-Tanomaru JM, Cerri PS. In vivo evaluation of the inflammatory response and IL-6 immunoexpression promoted by Biodentine and MTA Angelus. *Int Endod J*. 2016;49(2):145–153. doi:10.1111/iej.12435
18. Saraiva JA, Da Fonseca TS, Da Silva GF, et al. Reduced interleukin-6 immunoexpression and birefringent collagen formation indicate that MTA Plus and MTA Fillapex are biocompatible. *Biomed Mater*. 2018;13(3):035002. doi:10.1088/1748-605X/aaa1f5
19. Delfino MM, Guerreiro-Tanomaru JM, Tanomaru-Filho M, Sasso-Cerri E, Cerri PS. Immunoinflammatory response and bioactive potential of GuttaFlow bioseal and MTA Fillapex in the rat subcutaneous tissue. *Sci Rep*. 2020;10(1):7173. doi:10.1038/s41598-020-64041-0
20. Yaltirik M, Ozbas H, Bilgic B, Issever H. Reactions of connective tissue to mineral trioxide aggregate and amalgam. *J Endod*. 2004;30(2):95–99. doi:10.1097/00004770-200402000-00008
21. Zhang W, Peng B. Tissue reactions after subcutaneous and intraosseous implantation of iRoot SP, MTA and AH Plus. *Dent Mater J*. 2015;34(6):774–780. doi:10.4012/dmj.2014-271

22. Mena-Álvarez J, Rico-Romano C, Gutiérrez-Ortega C, Arias-Sanz P, Castro-Urda J. A comparative study of biocompatibility in rat connective tissue of a new mineral trioxide compound (Theracal) versus MTA and a bioactive G3 glass. *J Clin Med*. 2021;10(12):2536. doi:10.3390/jcm10122536
23. Chen Y, Yu Q, Xu CB. A convenient method for quantifying collagen fibers in atherosclerotic lesions by ImageJ software. *Int J Clin Exp Med*. 2017;10(10):14904-14910. https://e-century.us/files/ijcem/10/10/ijcem0059973.pdf. Accessed December 1, 2022.
24. Poggio C, Ceci M, Beltrami R, Dagna A, Colombo M, Chiesa M. Biocompatibility of a new pulp capping cement. *Ann Stomatol (Roma)*. 2014;5(2):69–76. PMID:25002921. PMCID:PMC4071365.
25. Murray PE, Godoy CG, Godoy FG. How is the biocompatibilty of dental biomaterials evaluated? *Med Oral Patol Oral Cir Bucal*. 2007;12(3):E258–E266. PMID:17468726.
26. Gomes Moura CC, Cunha TC, Crema VO, Dechichi P, Gabrielli Biffi JC. A study on biocompatibility of three endodontic sealers: Intensity and duration of tissue irritation. *Iran Endod J*. 2014;9(2):137–143. PMID:24688584. PMCID:PMC3961592.
27. Khalil WA, Abunasef SK. Can mineral trioxide aggregate and nanoparticulate EndoSequence root repair material produce injurious effects to rat subcutaneous tissues? *J Endod*. 2015;41(7):1151–1156. doi:10.1016/j.joen.2015.02.034
28. Rady D, Mubarak R, Abdel Moneim RA. Healing capacity of bone marrow mesenchymal stem cells versus platelet-rich fibrin in tibial bone defects of albino rats: An in vivo study. *F1000Res*. 2018;7:1573. doi:10.12688/f1000research.15985.1
29. Silva-Herzog D, Ramírez T, Mora J, et al. Preliminary study of the inflammatory response to subcutaneous implantation of three root canal sealers. *Int Endod J*. 2011;44(5):440–446. doi:10.1111/j.1365-2591.2011.01849.x
30. Zmener O, Guglielmotti MB, Cabrini RL. Biocompatibility of two calcium hydroxide-based endodontic sealers: A quantitative study in the subcutaneous connective tissue of the rat. *J Endod*. 1988;14(5):229–235. doi:10.1016/S0099-2399(88)80175-4
31. Giraud T, Jeanneau C, Rombouts C, Bakhtiar H, Laurent P, About I. Pulp capping materials modulate the balance between inflammation and regeneration. *Dent Mater*. 2019;35(1):24–35. doi:10.1016/j.dental.2018.09.008
32. Shahi S, Rahimi S, Lotfi M, Yavari H, Gaderian A. A comparative study of the biocompatibility of three root-end filling materials in rat connective tissue. *J Endod*. 2006;32(8):776–780. doi:10.1016/j.joen.2006.01.014
33. Koh ET, Torabinejad M, Pitt Ford TR, Brady K, McDonald F. Mineral trioxide aggregate stimulates a biological response in human osteoblasts. *J Biomed Mater Res*. 1997;37(3):432–439. doi:10.1002/(sici)1097-4636(19971205)37:3<432::aid-jbm14>3.0.co;2-d
34. Moretton TR, Brown CE Jr., Legan JJ, Kafrawy AH. Tissue reactions after subcutaneous and intraosseous implantation of mineral trioxide aggregate and ethoxybenzoic acid cement. *J Biomed Mater Res*. 2000;52(3):528–533. doi:10.1002/1097-4636(20001205)52:3<528::aid-jbm11>3.0.co;2-9
35. Sahin N, Saygili S, Akcay M. Clinical, radiographic, and histological evaluation of three different pulp-capping materials in indirect pulp treatment of primary teeth: A randomized clinical trial. *Clin Oral Investig*. 2021;25(6):3945–3955. doi:10.1007/s00784-020-03724-4
36. Camilleri J. Hydration characteristics of Biodentine and Theracal used as pulp capping materials. *Dent Mater*. 2014;30(7):709–715. doi:10.1016/j.dental.2014.03.012
37. Bakhtiar H, Nekoofar MH, Aminishakib P, et al. Human pulp responses to partial pulpotomy treatment with TheraCal as compared with Biodentine and ProRoot MTA: A clinical trial. *J Endod*. 2017;43(11):1786–1791. doi:10.1016/j.joen.2017.06.025
38. Hebling J, Rosetti Lessa FC, Nogueira I, Carvalho RM, Souza Costa CA. Cytotoxicity of resin-based light-cured liners. *Am J Dent*. 2009;22(3):137–142. PMID:19650592.
39. Jeanneau C, Laurent P, Rombouts C, Giraud T, About I. Light-cured tricalcium silicate toxicity to the dental pulp. *J Endod*. 2017;43(12):2074–2080. doi:10.1016/j.joen.2017.07.010
40. Hinata G, Yoshiba K, Han L, Edanami N, Yoshiba N, Okiji T. Bioactivity and biomineralization ability of calcium silicate-based pulp-capping materials after subcutaneous implantation. *Int Endod J*. 2017;50(Suppl 2):e40–e51. doi:10.1111/iej.12802
41. Rodríguez-Lozano FJ, López-García S, García-Bernal D, et al. Cytocompatibility and bioactive properties of the new dual-curing resin-modified calcium silicate-based material for vital pulp therapy. *Clin Oral Investig*. 2021;25(8):5009–5024. doi:10.1007/s00784-021-03811-0
42. Li X, Pedano MS, Li S, et al. Preclinical effectiveness of an experimental tricalcium silicate cement on pulpal repair. *Mater Sci Eng C Mater Biol Appl*. 2020;116:111167. doi:10.1016/j.msec.2020.111167
43. Koutroulis A, Kuehne SA, Cooper PR, Camilleri J. The role of calcium ion release on biocompatibility and antimicrobial properties of hydraulic cements. *Sci Rep*. 2019;9(1):1–10. doi:10.1038/s41598-019-55288-3
44. Nilsen BW, Jensen E, Örtengren U, Michelsen VB. Analysis of organic components in resin-modified pulp capping materials: Critical considerations. *Eur J Oral Sci*. 2017;125(3):183–194. doi:10.1111/eos.12347
45. Atsumi T, Ishihara M, Kadoma Y, Tonosaki K, Fujisawa S. Comparative radical production and cytotoxicity induced by camphorquinone and 9-fluorenone against human pulp fibroblasts. *J Oral Rehabil*. 2004;31(12):1155–1164. doi:10.1111/j.1365-2842.2004.01357.x
46. Engelmann J, Volk J, Leyhausen G, Geurtsen W. ROS formation and glutathione levels in human oral fibroblasts exposed to TEGDMA and camphorquinone. *J Biomed Mater Res B Appl Biomater*. 2005;75(2):272–276. doi:10.1002/jbm.b.30360
47. Elbanna A, Atta D, Sherief DI. In vitro bioactivity of newly introduced dual-cured resin-modified calcium silicate cement. *Dent Res J (Isfahan)*. 2022;19:1. doi:10.4103/1735-3327.336686
48. Ananda N, Ariawan D, Juniantito V. Effects of the *Hydnophytum formicarum* plant extract on collagen density, angiogenesis, wound length, and re-epithelialization in wound healing: Experimental study on rats. *Dent Med Probl*. 2022;59(1):67–73. doi:10.17219/dmp/140208
49. Kim I, Moon SO, Kim SH, Kim HJ, Koh YS, Koh GY. Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. *J Biol Chem*. 2001;276(10):7614–7620. doi:10.1074/jbc.M009705200
50. Yiğit U, Kırzıoğlu FY, Özmen Ö, Uğuz AC. Protective effects of caffeic acid phenethyl ester on the heart in experimental periodontitis against oxidative stress in rats. *Dent Med Probl*. 2021;58(3):335–341. doi:10.17219/dmp/132388
51. Giraud T, Jeanneau C, Bergmann M, Laurent P, About I. Tricalcium silicate capping materials modulate pulp healing and inflammatory activity in vitro. *J Endod*. 2018;44(11):1686–1691. doi:10.1016/j.joen.2018.06.009

Original paper

Increased inflammation, disturbed extracellular matrix remodeling and reduced parotid and submandibular salivary gland function in male Wistar rats with type 2 diabetes

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Abstract

Background. Insulin-independent type 2 diabetes mellitus (DM2) causes numerous organ complications, some of which involve the oral cavity. However, to date, the causes of reduced salivary secretion in patients with DM2 remain unclear.

Objectives. The present study is the first to evaluate the salivary inflammatory and anti-inflammatory profiles in the salivary glands of rats with DM2.

Material and methods. Twenty male Wistar Cmdb:WI rats were randomly divided into 2 groups: a control group and a DM2 group. Type 2 diabetes mellitus was induced by feeding the rats a high-fat diet and administering a single intraperitoneal injection of low-dose streptozotocin (35 mg/kg bw). Salivary gland function was evaluated using pilocarpine-stimulated salivary flow rate and α-amylase activity. The salivary inflammatory profile was assessed using a multiplex enzyme-linked immunosorbent assay (ELISA).

Results. Type 2 diabetes mellitus caused dysfunction of the parotid glands (PGs) and submandibular glands (SMGs), manifested by a reduction in the salivary flow rate and a decrease in salivary α-amylase activity. In DM2 rats, significantly higher levels of pro-inflammatory cytokines (interleukin (IL)-1α, tumor necrosis factor alpha (TNF-α), IL-6, IL-7, IL- 18), anti-inflammatory cytokines (IL-10), Th1 (IL-12) and Th17 (IL-17) cytokines, chemokines (monocyte chemoattractant protein (MCP)-1α/chemokine ligand 3 (CCL3), macrophage inflammatory protein (MIP)-1α/CCL3, regulated on activation, normal T-cell expressed and secreted chemokine (RANTES)/CCL5, MIP-3α/CCL20, growth-regulated oncogene (GRO)-1α/CCL1), and growth factors (macrophage colony-stimulating factor (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF) and vascular endothelial growth factor (VEGF)) were observed, as well as increased activity of salivary pro-inflammatory enzymes (myeloperoxidase (MPO), β-D-glucuronidase (GLU)). Evaluation of the inflammatory profile did not indicate the dominance of any branch of the immune system, with the PG being the main source of salivary cytokines, chemokines and growth factors. The activity of lysosomal exoglycosidases and extracellular matrix metalloproteinases (MMPs) involved in salivary gland remodeling was significantly higher in the PGs and SBGs of DM2 rats compared with controls.

Conclusions. Increased levels of salivary inflammatory biomarkers accompanied by decreased salivary secretory function may indicate the contribution of inflammation to salivary gland dysfunction. During the course of DM2, impairment predominantly affects PGs.

Keywords: inflammation, salivary glands, type 2 diabetes, glycoconjugates

Highlights

- Type 2 diabetes mellitus is associated with increased production of cytokines, chemokines and growth factors at both systemic (serum) and local (salivary gland) levels.
- The parotid gland represents the primary source of salivary cytokines, chemokines and growth factors.
- Salivary gland inflammation is associated with reduced secretory function.

Introduction

Diabetes mellitus (DM) constitutes a group of metabolic diseases characterized by elevated blood glucose levels. The discovery of the role of the pancreas and the hormone insulin has enabled a more accurate understanding of the causes of the disease and the development of more effective treatments.[1–3] Unfortunately, epidemiological studies indicate a continuous increase in the incidence of DM. Insulin-independent type 2 diabetes mellitus (DM2) is the most common form of DM, accounting for approx. 90–95% of all cases, and primarily affecting elderly or middle-aged individuals.[4] The disease is strongly influenced by lifestyle (e.g., dietary inadequacies, lack of exercise, low health awareness) and socioeconomic factors (e.g., limited access to medical care).[5] According to current knowledge, the underlying causes of DM2 are obesity and insulin resistance, i.e., reduced sensitivity of target tissues (liver, adipose tissue, skeletal muscle, or brain) to insulin.[6] Regardless of etiology, patients with DM2 are at risk of developing serious organ complications. These include specific diabetic complications such as retinopathy (eye damage), nephropathy (kidney damage) or neuropathy (damage to the nervous system), as well as non-specific cerebral, peripheral vascular and cardiac complications that also occur in individuals without DM.[7] Although the exact pathogenesis of these complications is not fully understood, their common feature is an active inflammatory process.[8]

The oral cavity is also susceptible to DM2-related complications.[9] Patients with DM2 are more likely to develop periodontitis, which may progress to alveolar bone loss and gingival attachment atrophy. Several studies have shown that periodontitis-induced bacteremia increases the production of pro-inflammatory cytokines and reactive oxygen species (ROS), which can exacerbate insulin resistance and impair metabolic control.[9,10] Therefore, DM negatively affects periodontal status, while periodontitis may contribute to disease progression.[10] Impaired saliva secretion represents another clinical problem in patients with DM.[11] In these individuals, the qualitative composition of saliva and the secretory activity of salivary glands are changed.[12] Reduction in the secretion of unstimulated and stimulated saliva (hyposalivation) increases the incidence of caries and periodontal disease.[10] In addition, dry oral mucous membranes (xerostomia) and hyposalivation significantly increase the risk of mechanical damage, aphthous ulcers and fungal infections, such as candidiasis.[13] Burning mouth syndrome and oral-facial syndrome are also associated with xerostomia and hyposalivation and are frequently reported by patients with DM.[14] Although antidiabetic medications may influence salivary secretion, the mechanisms underlying hyposalivation in patients with DM2 remain unclear.

Saliva constitutes the fluid environment of the oral cavity and consists of salivary gland secretions, gingival crevicular fluid, exfoliated epithelial cells, leukocytes, and microorganisms. The major salivary glands play a key role in saliva secretion.[15] The parotid glands (PGs) produce approx. 20% of total salivary volume and secrete serous saliva with high water and enzyme content. The largest volume of saliva (70%) is excreted by the submandibular glands (SMGs). This secretion contains enzymes and mucus and, thus, has a medium density. In contrast, saliva secreted by the sublingual glands accounts for only 5% of total salivary secretion.[15] In our previous study, we demonstrated that ceramide accumulation in the salivary glands of rats with obesity and insulin resistance induces oxidative stress, inflammation and apoptosis by affecting pro-oxidant enzymes and the mitochondrial respiratory chain.[16–18] Importantly, ROS production, nitration of salivary proteins and peroxidation of membrane lipids were more pronounced in the PGs of rats with insulin resistance induced by high-fat or high-carbohydrate diets.[18,19] However, studies using DM2 models remain limited. Although insulin resistance is a major factor in the development of DM2, progressive deterioration of insulin sensitivity of target tissues ultimately leads to long-term hyperglycemia and inflammation, resulting in overt DM2 and numerous organ complications. Micro- and macroangiopathic complications are the main cause of reduced quality of life and increased mortality in patients with DM2.[20,21] Given the central role of inflammation in DM2 progression in target tissues, it is plausible that inflammatory mechanisms also contribute to salivary gland hypofunction, similar to other systemic inflammatory disorders, such as Sjögren's syndrome,[22] chronic circulatory insufficiency,[23] Hashimoto's thyroiditis,[24] or psoriasis.[25] Therefore, in the present study, we aimed to assess the inflammatory

profile of the PGs and SMGs in rats with DM2, as well as the activity of lysosomal enzymes and extracellular matrix metalloproteinases (MMPs) involved in salivary gland tissue remodeling.[26] Our findings may contribute to a better understanding of the causes of salivary gland hypofunction in DM2, which significantly impairs patients' quality of life.

Material and methods

Study sample

The study was conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" by the National Academy of Sciences, and was approved by the Local Ethical Committee for Animal Experiments of the University of Warmia and Mazury in Olsztyn, Poland (approval No. 62/2021).

The experiment was performed on 20 male Wistar Cmdb:WI rats from a non-breeding, genetically heterogeneous outbred herd, with an initial body weight of approx. 180–200 g (5–8 weeks old). Only male rats were included to eliminate potential confounding effects of sex hormones.[27] The study was performed at the Experimental Medicine Centre of the Medical University of Bialystok, Poland. Cages for the animals were individually ventilated, ensuring the highest sanitary rigor. The rats were housed under standard laboratory conditions (21 ±2°C, 12 h light/12 h dark cycle, 60–80% humidity), with free access to water and food.

After a one-week acclimatization period, the rats were randomly divided into 2 equal groups (Fig. 1):

– C (control group (n = 10)) – rats fed a standard diet (D12450J; Research Diets, New Brunswick, USA) containing 10 kcal% fat, 70 kcal% carbohydrates and 20 kcal% protein for 10 weeks and receiving a single intraperitoneal injection of citrate buffer (pH 4.5) at week 4;

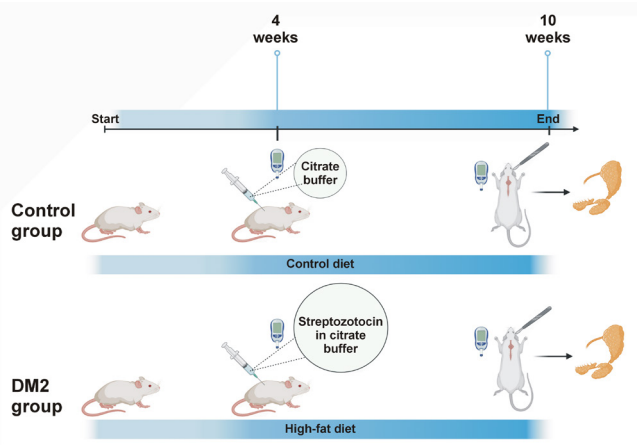


Fig. 1. Experimental design (created with BioRender.com)
DM2 – type 2 diabetes mellitus.

– DM2 (type 2 diabetes group (n = 10)) – rats fed a high-fat diet (D12492; Research Diets) containing 60 kcal% fat, 20 kcal% carbohydrates and 20 kcal% protein for 10 weeks and receiving a single intraperitoneal injection of streptozotocin (35 mg/kg bw) in citrate buffer (pH 4.5) at week 4.

Induction of DM2 by a high-fat diet and a single intraperitoneal injection of low-dose streptozotocin constitutes a well-established experimental model.[28–30] Type 2 diabetes mellitus was confirmed 72 h after the administration of streptozotocin by measuring fasting blood glucose levels.[3,31] The measurements were performed using a strip glucose meter (ACCU-CHEK® Active; Roche, Mannheim, Germany) in tail blood obtained by needle puncture or tail-tip incision, depending on the anatomical conditions of the animal. Caudal blood glucose levels were ≥200 mg/dL in all rats from the study group.[3,31]

After 10 weeks, the rats were fasted for 6 h. The animals had their tail blood drawn to measure glucose concentration. Then, pentobarbital sodium (80 mg/kg bw) was administered intraperitoneally. To assess salivary secretion, rats were injected intraperitoneally with pilocarpine hydrochloride (5 mg/kg bw). After 5 min, a lignin swab was placed under the tongue.[32,33] Following another 5 min, the swab was removed, and the rate of saliva production was calculated based on the difference between final and initial swab weights, assuming that 1 g of saliva corresponds to 1 mL.

Animals under anesthesia had blood drawn from the abdominal aorta and left ventricle. Blood samples were centrifuged at 3,000 × g (4°C, 10 min) to obtain serum. The salivary glands (SMGs and PGs) were excised by experienced technicians, cleaned and weighed. After skin incision and muscle dissection, glands were carefully visualized, excised with surgical scissors and rinsed in phosphate buffered saline (PBS (pH 7.0)). Excess adipose tissue was removed if present.[32,33] The salivary gland index was calculated as the ratio of gland weight to total body weight and expressed as a percentage.[32] Tissues were snap-frozen in liquid nitrogen and stored at –80°C for no longer than 6 months until analysis.

Biochemical determinations

Unless stated otherwise, all reagents were purchased from Sigma-Aldrich (Nümbrecht, Germany) or Sigma-Aldrich (St. Louis, USA). Total protein concentration was measured using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, USA), according to the manufacturer's instructions. The absorbance/fluorescence was measured using a multimode microplate reader (Infinite® M200 PRO; Tecan Group Ltd., Männedorf, Switzerland).

Salivary gland homogenates were analyzed for: (1) salivary α-amylase activity (Enzyme Commission (EC)

number 3.2.1.1); (2) concentrations of pro-inflammatory cytokines (interleukin (IL)-1α, IL-1β, tumor necrosis factor alpha (TNF-α), IL-6, IL-7, IL-18), anti-inflammatory cytokines (IL-10, IL-13), Th1 cytokines (interferon gamma (INF-γ), IL-2, IL-12), Th2 cytokines (IL-4, IL-5), Th17 cytokines (IL-17), chemokines (monocyte chemoattractant protein (MCP)-1α/chemokine ligand (CCL)2, macrophage inflammatory protein (MIP)-1α/CCL3, regulated on activation, normal T-cell expressed and secreted chemokine (RANTES)/CCL5, MIP-3α/CCL20, growth-regulated oncogene (GRO)-lα/CXCL1), growth factors (granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF)), and other DM2 biomarkers (ghrelin, glucagon, leptin, IL-12p40, MIP-2); (3) pro-inflammatory enzyme activity (β-D-glucuronidase (GLU, EC 3.2.1.31) and myeloperoxidase (MPO, EC 1.11.1.7)); (4) activity of enzymes involved in salivary gland remodeling (lysosomal exoglycosidases: N-acetyl-β-D-hexosaminidase (HEX, EC 3.2.1.52), its isoenzymes (HEX A and HEX B), β-D-galactosidase (GAL, EC 3.2.1.23), α-D-mannosidase (MAN, EC 3.2.1.24), α-D-fucosidase (FUC, EC 3.2.1.51), and extracellular MMPs: MMP-2 (EC 3.4.24.24), MMP-9 (EC 3.24.35)); (5) pro-apoptotic enzyme activity (caspase-3, EC 3.4.22.56) (Fig. 2).

Left salivary glands were used for α-amylase, MPO and inflammatory profile analyses, while right glands were used for other assays. Tissue homogenization

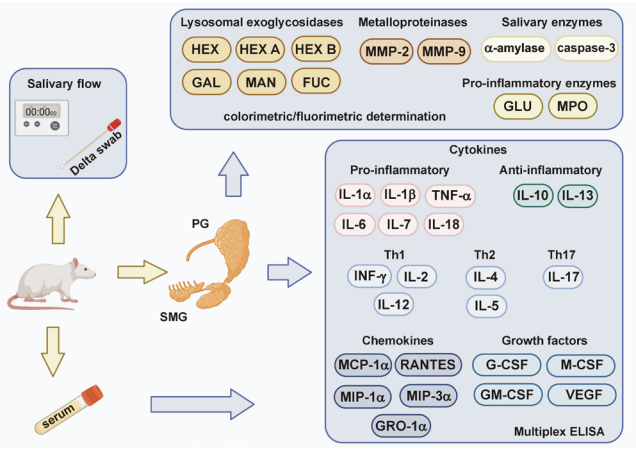


Fig. 2. Overview of biochemical determinations performed in the study (created with BioRender.com)

ELISA – enzyme-linked immunosorbent assay; FUC – α-D-fucosidase; GAL – β-D-galactosidase; G-CSF – granulocyte colony stimulating factor; GLU – β-D-glucuronidase; GM-CSF – granulocyte macrophage colony stimulating factor; GRO – growth-regulated oncogene; HEX – N-acetyl-β-D-hexosaminidase; IL – interleukin; INF-γ – interferon gamma; MAN – α-D-mannosidase; MCP – monocyte chemoattractant protein; M-CSF – macrophage colony stimulating factor; MIP – macrophage inflammatory protein; MMP – matrix metalloproteinase; MPO – myeloperoxidase; PG – parotid gland; RANTES – regulated on activation, normal T-cell expressed and secreted chemokine; SMG – submandibular gland; TNF-α – tumor necrosis factor alpha; VEGF – vascular endothelial growth factor.

was performed on the day of analysis. Salivary glands were slowly thawed on ice, then divided into smaller pieces and diluted (1:10, w:v).[16,17] For the assessment of α-amylase activity, tissues were homogenized in 0.02 M of phosphate buffer (pH 6.9), containing 6.7 mM of NaCl,[34,35] for the assessment of MPO activity – in hexadecyltrimethylammonium bromide (HTAB) buffer (0.5% HTAB in 50 mM of phosphate buffer (pH 6.0)),[36] for the evaluation of acid lysosomal glycosidases – in 0.15 M of KCl containing 0.2% Triton X-100,[35] for the analysis of MMPs activity – in TNC buffer (pH 7.5) consisting of 50 mM of tris(hydroxymethyl)aminomethane (Tris), 0.15 M of NaCl, 10 mM of $CaCl_2$, and 0.02% NaN_3,[37] and for the assessment of caspase-3 activity, tissues were homogenized in caspase extraction buffer (pH 7.5) containing 25 mM of 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 5 mM of $MgCl_2$, 1 mM of ethylenediaminetetraacetic acid (EDTA), 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), and 10 μg/mL of aprotinin.[38] Protease inhibitor (cOmplete™ Mini; Roche) (1 tablet/10 mL of homogenization buffer) and antioxidant (100 μL of 0.5 M butylated hydroxytoluene in acetonitrile/10 mL of homogenization buffer) were added to the samples. Samples were homogenized on ice using a glass homogenizer (Omni TH; Omni International, Kennesaw, USA). The resulting suspension was subjected to ultrasound using a cell sonifier (UP 400S; Hielscher Ultrasonics, Teltow, Germany) (1,800 J/sample, 20 s × 3). After disruption of the cell membranes, the homogenate was incubated on ice for 30 min and centrifuged at 5,000 × g (4°C, 30 min).[16,17] Supernatants were collected for analysis. To assess the inflammatory profile, tissues were homogenized using the ready-to-use Bio-Plex® Cell Lysis Kit (Bio-Rad Laboratories, Inc., Hercules, USA), according to the manufacturer's instructions.

To assess salivary secretory function, in addition to measuring the salivary flow rate, α-amylase activity was assessed spectrophotometrically using 3,5-dinitrosalicylic acid (POCH, Gliwice, Poland) as a substrate.[34,35] The results were standardized per mg of total protein and presented in mg/mg protein.

Cytokine, chemokine and growth factor concentrations in salivary glands and blood serum were determined using the Bio-Plex™ Rat Cytokine, Chemokine and Growth Factor Assay kit (Bio-Rad Laboratories, Inc.). The principle of the assay is similar to the sandwich enzyme-linked immunosorbent assay (ELISA) method, in which antibodies against a specific biomarker are covalently bound to magnetic beads. The conjugated beads react with a sample containing the selected biomarker. A series of washes are performed to remove the unbound protein. A biotinylated detection antibody is then added to form a sandwich complex. The final complex is formed by the addition of a streptavidin–phycoerythrin conjugate, and the readout is performed using a specialized plate reader

(Bio-Plex 200; Bio-Rad Laboratories, Inc.). The results were standardized per mg of total protein and present in ng/mg protein.

The activity of GLU in salivary glands was measured spectrophotometrically at 405 nm using 4-nitrophenyl-β-D-glucuronide as a substrate.[35] The results were standardized per mg of total protein and presented in pKat/mg protein.

The activity of MPO was measured at 450 nm using sulfanilamide, ortho-dianisidine dihydrochloride, hexadecyltrimethylammonium, and H_2O_2.[36] The results were standardized per mg of total protein and presented in mU/mg protein.

Lysosomal exoglycosidase activities were determined at 405 nm using 4-nitrophenyl-N-acetyl-β-D-glucosaminide (for HEX, HEX A and HEX B), 4-nitrophenyl-N-acetyl-β-D-galactopyranoside (for GAL), 4-nitrophenyl-N-acetyl-α-D-mannopyranoside (for MAN), and 4-nitrophenyl-N-acetyl-α-D-fucopyranoside (for FUC[35]). The HEX isoenzymes were determined after the heat denaturation of the isoform A. The results were standardized per mg of total protein and presented in pKat/mg protein.

The activity of MMP-2 and MMP-9 in salivary glands was measured spectrofluorimetrically using the MCA-Pro-Leu-Gly~Leu-Dpa(Dnp)-Ala-Arg-NH_2 substrate, where MCA is the fluorophore (7-methoxycoumarin-4-yl) acetate, Dnp is the quencher dinitrophenyl, and the tilde (~) represents the peptide-bond target of hydrolysis.[37] The results were standardized per mg of total protein and presented in AFU/mg protein.

The activity of caspase-3 was measured at 405 nm using Ac-Asp-Glu-Val-Asp-p-nitroanilide as a substrate.[38] The results were standardized per mg of total protein and expressed in mU/mg protein.

Statistical analysis

The GraphPad Prism 9.5.1 software (GraphPad Software Inc., La Jolla, USA) was used to process the statistical data. The normality of the distribution was checked using the Shapiro–Wilk test, while the homogeneity of variance was evaluated using the Levene's test. A two-way ANOVA with factors including salivary gland type and presence of DM2, followed by Tukey's honest significant difference (HSD) post hoc test, was used for comparisons. The results are presented as mean (*M*) ± standard error of the mean (*SEM*). The level of statistical significance was set at $p < 0.05$. Multiplicity-adjusted *p*-value was also calculated. Student's *t*-test was used to compare the 2 groups. Since the data showed a normal distribution, the obtained results were correlated using Pearson's test. Multivariate regression analysis was used to assess the relationship between salivary secretory function and the biomarkers assessed, using the salivary secretion rate and salivary α-amylase activity as independent variables. Confidence intervals of 95% were reported along with regression parameters.

The sample size was calculated a priori based on our previous experiments. Type I error (α) of 0.05 and statistical power (type II error) of 0.8 were considered. The minimum number of rats in one group was 8, and the analysis was performed on 10 subjects.

Results

Impaired salivary function in diabetic rats

Fasting blood glucose levels were significantly elevated in the DM2 group compared to the control group. All rats in the DM2 group had fasting glucose levels above 200 mg/dL, confirming the onset of diabetes.

Body weight was significantly higher in the study group than in the control group. Although salivary gland mass did not differ between the groups, the PG index was significantly lower in rats with DM2 compared with controls. The salivary secretion rate was also significantly reduced in the DM2 group. Furthermore, α-amylase activity, used as an indicator of salivary secretory function, was significantly lower in both PGs and SMGs of diabetic rats compared with controls (Table 1).

Table 1. General characteristics and salivary gland function parameters of rats with type 2 diabetes mellitus (DM2) and the control group

Variable	Control group (*n* = 10)	DM2 group (*n* = 10)	*p*-value
Fasting glucose [mg/dL]	90 ±2	295 ±16	<0.0001*
Body weight [g]	403 ±7	489 ±12	<0.0001*
PG weight [g]	0.09 ±0.008	0.08 ±0.005	0.227
SMG weight [g]	0.26 ±0.005	0.28 ±0.020	0.070
PG index [%]	0.02 ±0.002	0.02 ±0.001	0.010*
SMG index [%]	0.06 ±0.001	0.06 ±0.002	0.072
Salivary flow rate [mL/min]	0.17 ±0.007	0.12 ±0.010	0.002*
α-amylase in PGs [mg/mg protein]	11.4 ±0.96	7.2 ±0.96	0.007*
α-amylase in SMGs [mg/mg protein]	10.6 ±1.90	4.7 ±0.64	0.009*

Data presented as mean (*M*) ± standard error of mean (*SEM*). The salivary gland (submandibular gland (SMG) or parotid gland (PG)) index was calculated as the ratio of gland weight to total body weight.
* statistically significant ($p < 0.05$, Student's *t*-test).

Salivary pro-inflammatory cytokines

Concentrations of IL-1α standardized to total protein were significantly higher in the PGs than in the SMGs in both control and DM2 groups. IL-1α levels were significantly elevated in both glands in rats with DM2 compared with controls. IL-1β content was also significantly higher in the PGs than in the SMGs in both groups. TNF-α concentrations were higher in the PGs than in the SMGs in both groups and were significantly increased in DM2 rats compared with controls. The IL-6 content in both the PGs and SMGs was significantly elevated in the DM2 group. IL-7 concentrations were higher in the PGs than in the SMGs in both groups and were further increased in diabetic rats. In contrast, IL-18 levels were higher in the SMGs than in the PGs in both groups. In both glands, IL-18 concentrations were significantly higher in rats with DM2 than in the control group (Fig. 3).

Salivary anti-inflammatory cytokines

IL-10 concentrations were significantly higher in the PGs than in the SMGs and were increased in both salivary glands in the DM2 group compared with controls (Fig. 3).

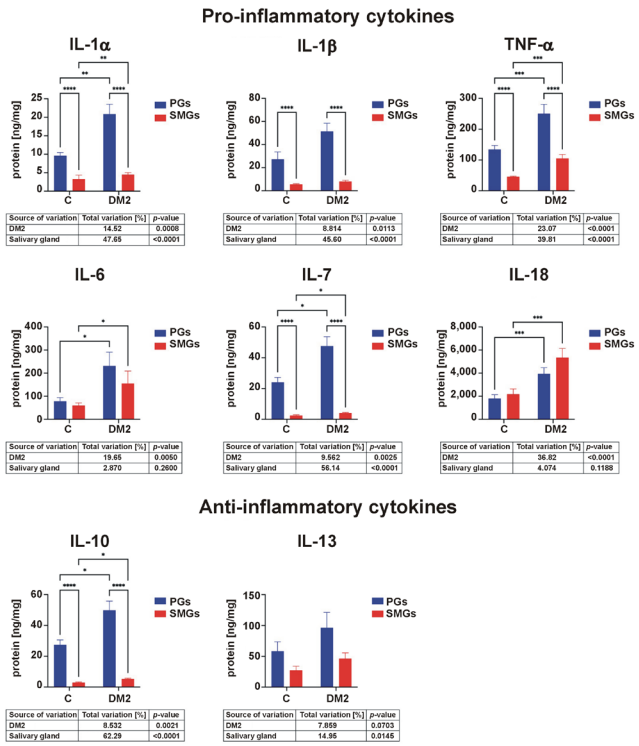


Fig. 3. Pro-inflammatory and anti-inflammatory cytokine levels in the parotid glands (PGs) and submandibular glands (SMGs) of rats with type 2 diabetes mellitus (DM2) and control group (C), analyzed using multiplex enzyme-linked immunosorbent assay (ELISA)

Comparisons were performed using two-way ANOVA with factors including salivary gland type and presence of DM2, followed by Tukey's honest significant difference (HSD) post hoc test. Results are presented as mean (*M*) ± standard error of mean (*SEM*). Multiplicity-adjusted *p*-values were calculated.
* $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$; **** $p < 0.0001$.

Salivary Th1 cytokines

Concentrations of IL-12 were significantly higher in the PGs than in the SMGs and were elevated in both glands in rats with DM2 compared with controls. IL-2 levels were higher in the PGs than in the SMGs in both groups (Fig. 4).

Salivary Th2 cytokines

IL-4 and IL-5 concentrations were elevated in the PGs compared to the SMGs in both control and DM2 groups (Fig. 4).

Salivary Th17 cytokines

Levels of IL-17 were significantly higher in the PGs than in the SMGs in both groups. In addition, IL-17 concentrations in both glands were significantly elevated in rats with DM2 compared with controls (Fig. 4).

Salivary chemokines

MCP-1α/CCL2 levels were significantly higher in the PGs than in the SMGs in both groups and were significantly increased in DM2 rats. Levels of MIP-1α/CCL3 were

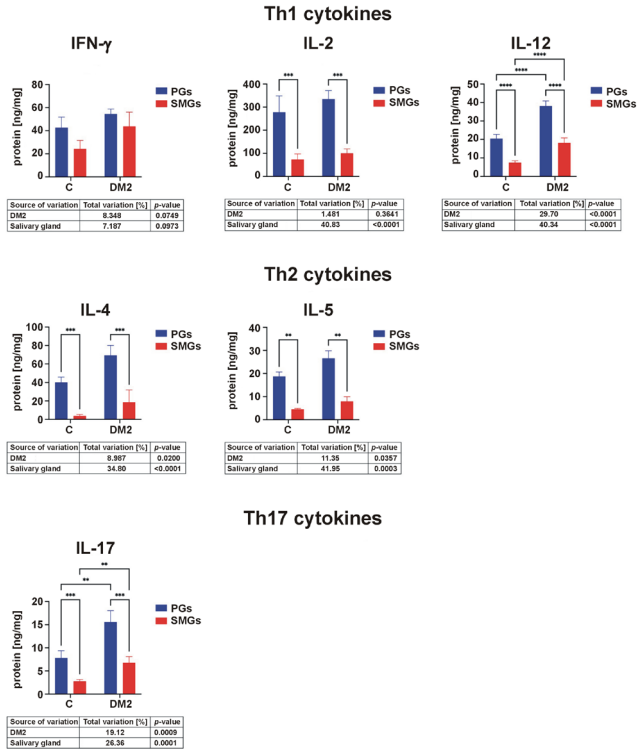


Fig. 4. Th1, Th2 and Th17 cytokine levels in the parotid glands (PGs) and submandibular glands (SMGs) of rats with type 2 diabetes mellitus (DM2) and control group (C), analyzed using multiplex enzyme-linked immunosorbent assay (ELISA)

Comparisons were performed using two-way ANOVA, followed by Tukey's HSD post hoc test. Results are presented as *M* ±*SEM*. Multiplicity-adjusted *p*-values were calculated.
** $p < 0.005$; *** $p < 0.0005$; **** $p < 0.0001$.

elevated in both glands in the DM2 group. RANTES/CCL5 concentrations were higher in the PGs than in the SMGs in both groups and were significantly increased in diabetic rats. Levels of MIP-3α/CCL20 were higher in the PGs than in the SMGs for both groups and were elevated in rats with DM2. GRO-1α/CXCL1 concentrations were higher in the PGs than in the SMGs in both groups and were significantly increased in both glands in the DM2 group (Fig. 5).

Salivary growth factors

M-CSF concentrations were significantly higher in the SMGs than in the PGs in both groups and were elevated in diabetic rats. GM-CSF levels were higher in the PGs than in the SMGs in both groups and were significantly increased in rats with DM2. The concentration of VEGF was higher in both PGs and SMGs in the DM2 group compared with controls (Fig. 5).

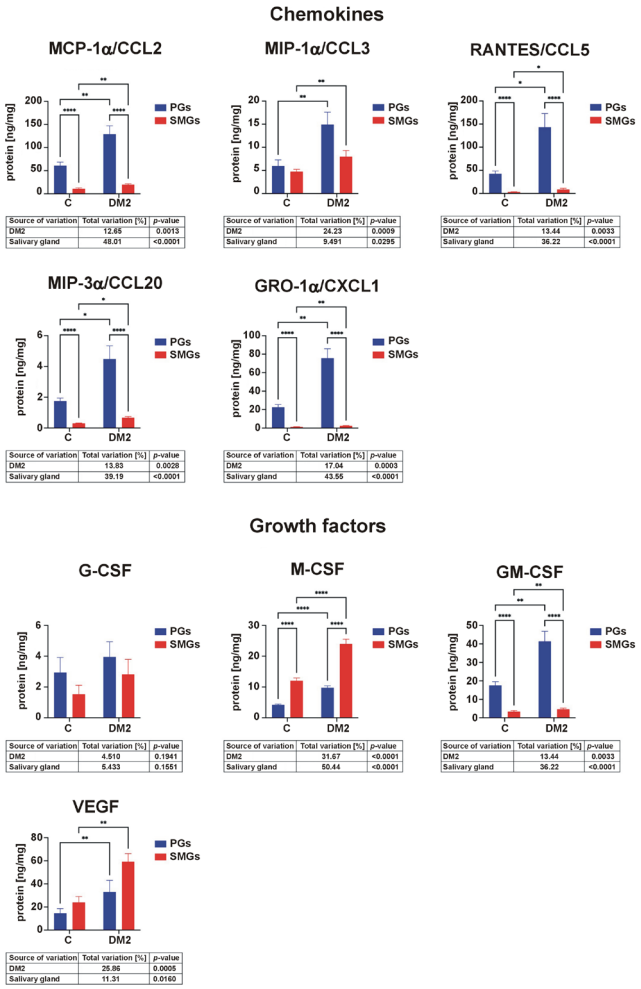


Fig. 5. Chemokine and growth factor levels in the parotid glands (PGs) and submandibular glands (SMGs) of rats with type 2 diabetes mellitus (DM2) and control group (C), analyzed using multiplex enzyme-linked immunosorbent assay (ELISA)

Comparisons were performed using two-way ANOVA, followed by Tukey's HSD post hoc test. Results are presented as *M* ±*SEM*. Multiplicity-adjusted *p*-values were calculated.
* $p < 0.05$; ** $p < 0.005$; **** $p < 0.0001$.

Other biomarkers

Concentrations of ghrelin, glucagon, leptin, IL-12p40, and MIP-2 were below the detection level in both salivary glands of control and DM2 rats.

Blood inflammatory and anti-inflammatory profiles

Serum concentrations of pro-inflammatory cytokines (IL-lα, IL-1β, TNF-α) were significantly higher in the DM2 group than in controls. In contrast, IL-10 levels were significantly lower in diabetic rats. Th1 cytokines (IFN-γ, IL-2, IL-12) were significantly elevated in the serum of rats with DM2. Concentrations of Th2 cytokine (IL-6) were higher in the DM2 group, whereas Th17 cytokine (IL-17) levels were significantly reduced in the study group. RANTES/CCL5 and VEGF concentrations were also significantly increased in diabetic rats. Other parameters did not differ significantly between the groups (Table S1 (available on request from the corresponding author)).

Pearson's correlation analysis revealed no consistent relationship between salivary and serum cytokine levels. Only IL-6 concentrations in the SMGs correlated positively with their plasma content in the control rats, while IL-7, MCP-1α and GRO-1α concentrations correlated positively between the PGs and serum in diabetic rats (Table S2).

Salivary pro-inflammatory enzymes

The activity of GLU was significantly higher in the DM2 group than in controls in both salivary glands. The activity of MPO was also elevated in PGs and SMGs of diabetic rats (Fig. 6).

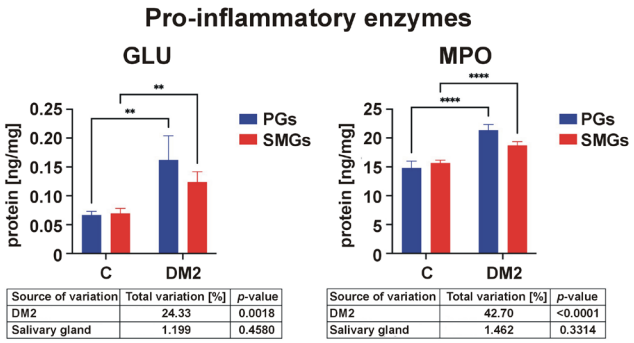


Fig. 6. Pro-inflammatory enzyme activity in the parotid glands (PGs) and submandibular glands (SMGs) of rats with type 2 diabetes mellitus (DM2) and control group (C), analyzed using colorimetric assays

Comparisons were performed using two-way ANOVA, followed by Tukey's HSD post hoc test. Results are presented as *M* ±*SEM*. Multiplicity-adjusted *p*-values were calculated.
** $p < 0.005$; **** $p < 0.0001$.

Salivary exoglycosidases

Inflammation disrupts salivary extracellular matrix (ECM) remodeling. Glycoconjugates, i.e., molecules made out of one or more oligosaccharide or polysaccharide chains which are linked by covalent bonds to a protein or lipid by a N- or O-glycosidic bond, are key components of the salivary ECM. These include glycoproteins, proteoglycans or sphingolipids.[39,40] The enzymes responsible for the metabolic turnover of salivary glycoconjugates are lysosomal exoglycosidases. Salivary exoglycosidases hydrolyze single monosaccharides from the non-reducing ends of oligosaccharides. In this group of enzymes, we distinguish HEX and its isoforms (HEX A and HEX B), as well as the less active GAL, MAN and FUC.[35]

The activity of HEX and HEX B was significantly higher in DM2 rats than in controls in both glands. In contrast, FUC activity was reduced in diabetic rats (Fig. 7).

Salivary extracellular matrix metalloproteinases

In addition to lysosomal exoglycosidases, salivary gland remodeling is also contingent upon enzymes from the MMP group. Matrix metalloproteinases belong to proteolytic enzymes that digest ECM components and basement membranes. Among these enzymes, MMP-2 and MMP-9 are most commonly associated with metabolic diseases.[41] Due to their structural similarity and ability to degrade gelatin, MMP-2 and MMP-9 belong to the gelatinase group. Compared to other MMPs, they exhibit a high activity within the salivary glands.[26]

Both MMP-2 and MMP-9 activities were significantly higher in rats with DM2 than in controls in both salivary glands (Fig. 7).

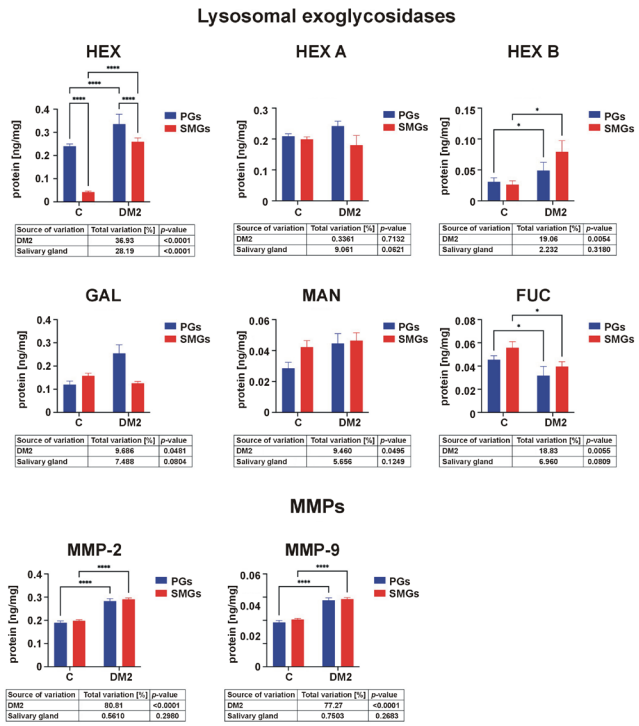


Fig. 7. Lysosomal exoglycosidase and extracellular matrix metalloproteinase (MMP) activities in the parotid glands (PGs) and submandibular glands (SMGs) of rats with type 2 diabetes mellitus (DM2) and control group (C), analyzed using colorimetric and fluorimetric assays

Comparisons were performed using two-way ANOVA, followed by Tukey's HSD post hoc test. Results are presented as *M* ±*SEM*. Multiplicity-adjusted *p*-values were calculated.
* $p < 0.05$; **** $p < 0.0001$.

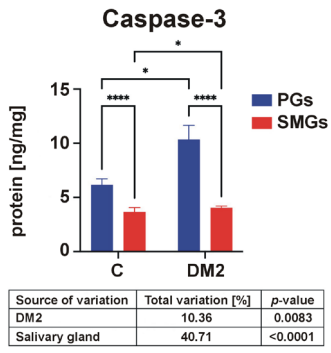
Salivary caspase-3

Inflammation and impaired ECM remodeling may promote apoptosis in salivary gland cells. Therefore, caspase-3 activity, the key enzyme responsible for the execution of apoptosis, was assessed.[42] Caspase-3 activity was significantly higher in the PGs than in the SMGs in both control and DM2 groups and was significantly increased in diabetic rats (Fig. 8).

Correlation analysis

Pearson's correlation analysis was used to measure the strength and direction of linear relationships between inflammatory biomarkers and salivary gland secretory function. The results are presented in Tables S3 and S4.

In the control group, only a limited number of correlations was observed between the salivary inflammatory profile and secretory function. In the PGs in the control group, salivary α-amylase activity correlated positively with IL-1β ($r = 0.72$, $p = 0.02$), MCP-1α/CCL3 ($r = 0.73$, $p = 0.016$) and RANTES/CCL5 ($r = 0.65$, $p = 0.043$) (Table S3). In the SMGs, the salivary secretion rate correlated positively with HEX ($r = 0.74$, $p = 0.014$) and MAN ($r = 0.31$, $p = 0.014$). Furthermore, salivary α-amylase activity was negatively correlated with MPO activity ($r = -0.68$, $p = 0.031$) and positively correlated with caspase-3 activity ($r = 0.94$, $p = 0.025$) (Tables S3 and S4).



Source of variation	Total variation [%]	*p*-value
DM2	10.36	0.0083
Salivary gland	40.71	<0.0001

Fig. 8. Caspase-3 activity in the parotid glands (PGs) and submandibular glands (SMGs) of rats with type 2 diabetes mellitus (DM2) and control group (C), analyzed using colorimetric assays

Comparisons were performed using two-way ANOVA, followed by Tukey's HSD post hoc test. Results are presented as *M* ±*SEM*. Multiplicity-adjusted *p*-values were calculated.
* $p < 0.05$; **** $p < 0.0001$.

Although the study is observational in nature, the presence of a potential association between inflammation and salivary gland dysfunction in rats with DM2 may be confirmed by the negative correlations between salivary cytokines, the salivary secretion rate and salivary α-amylase activity. In the PGs of rats with DM2, the salivary secretion rate correlated negatively with IL-1α ($r = -0.74$, $p = 0.015$), IL-7 ($r = -0.68$, $p = 0.031$), IL-10 ($r = -0.69$, $p = 0.026$), IL-2 ($r = -0.87$, $p = 0.005$), IL-12 ($r = -0.77$, $p = 0.01$), IL-4 ($r = -0.69$, $p = 0.027$), IL-5 ($r = -0.86$, $p = 0.002$), MCP-1α/CCL2 ($r = -0.67$, $p = 0.035$), and VEGF ($r = -0.67$, $p = 0.035$). Salivary α-amylase activity in the PGs correlated negatively with IL-1β ($r = -0.74$, $p = 0.022$), TNF-α ($r = -0.79$, $p = 0.011$), IL-6 ($r = -0.78$, $p = 0.013$), IL-7 ($r = -0.86$, $p = 0.003$), IL-10 ($r = -0.82$, $p = 0.007$), IL-12 ($r = -0.69$, $p = 0.041$), IL-4 ($r = -0.79$, $p = 0.012$), IL-5 ($r = -0.72$, $p = 0.03$), MCP-1α/CCL2 ($r = -0.85$, $p = 0.004$), and GM-CSF ($r = -0.84$, $p = 0.004$) (Tables S3 and S4).

In the SMGs of DM2 rats, the salivary secretion rate correlated negatively with M-CSF concentration ($r = -0.66$, $p = 0.037$). Salivary α-amylase activity was negatively correlated with TNF-α ($r = -0.71$, $p = 0.034$), GLU activity ($r = -0.72$, $p = 0.028$) and HEX B activity ($r = -0.65$, $p = 0.04$). In contrast, α-amylase activity correlated positively with G-CSF concentration ($r = 0.67$, $p = 0.034$), HEX activity ($r = 0.65$, $p = 0.042$) and HEX A activity ($r = 0.72$, $p = 0.019$) (Tables S3 and S4).

Regression analysis

Multiple regression analysis was performed to evaluate the relationship between multiple independent (explanatory) variables and the dependent (response) variable. The salivary production rate and α-amylase activity were used as predictors. The results are presented in Table S5.

Multivariate linear regression analysis revealed that the content of pro-inflammatory cytokines (IL-lα) in the PGs was negatively correlated with the saliva secretion rate, as was the concentration of TNF-α in the SMGs. The content of anti-inflammatory cytokines (IL-10) in the SMGs was also negatively associated with the rate of saliva production, similarly to the concentration of Th1 cytokines (IL-12). The content of Th2 cytokines (IL-6) in the PGs was negatively associated with α-amylase activity. Chemokine levels, including MIP-1α/CCL3 and MIP-3α/CCL20 in the SMGs, were negatively correlated with the salivary secretion rate, while RANTES/CCL5 and GRO-1α/CXCL1 in the PGs were negatively associated with the salivary secretion rate. The content of growth factors (M-CSF) in both SMGs and PGs was negatively associated with salivary secretion. The concentration of VEGF in the SMGs exhibited a negative correlation with the salivary secretion rate. Pro-inflammatory enzyme (GLU) activity in the SMGs was negatively associated with the rate of saliva production, whereas MPO activity was negatively associated with α-amylase activity. The activity of salivary exoglycosidases in the SMGs was negatively correlated with α-amylase activity. Furthermore, the activity of salivary MMPs (MMP-2, MMP-9) in the SMGs was negatively associated with the salivary secretion rate (Table S5).

Discussion

Diabetes mellitus constitutes a group of metabolic diseases resulting from defects in insulin secretion and/or insulin action. Chronic hyperglycemia impairs the function of many systems and organs, including the oral cavity, leading to salivary secretory dysfunction.[43] Xerostomia is observed in up to 80% of patients with DM and is associated with reduced resting and stimulated salivary secretion.[44] This condition significantly reduces patients' quality of life by causing speech and digestive disorders and by promoting the development of oral diseases.[45] However, the causes of salivary hypofunction in patients with DM2 are not fully understood.[46,47] It is presumed that autonomic nerve dysfunction and microcirculatory disturbances may reduce the responsiveness of salivary secretory cells to neural or hormonal stimulation.[48,49] Undoubtedly, oxidative stress and carbonyl stress contribute to salivary hypofunction through glycoxidative modifications of salivary glands.[50–52]

Large salivary glands, namely the PGs and SMGs, produce more than 90% of total salivary volume.[15] The salivary gland epithelium has a highly branched tubular structure composed of terminal units/acini that secrete saliva, a network of interlobular, granular (in rodents), striated, and excretory ducts that serve to modify and transport saliva, and a myoepithelium that supports the excretion of saliva from the acini through the ducts.[53] The activation of muscarinic or α-adrenergic receptors initiates salivary secretion, while the stimulation of β-adrenergic receptors induces protein exocytosis.[54] In patients with DM2, degenerative and hypertrophic changes in salivary glands resulting from diabetic microangiopathy have been reported. A reduction in tubular structures, their concomitant hypertrophy, and decreased density of α- and β-adrenergic receptors have been observed, leading to hyposalivation and impaired protein secretion.[55–57] In the present study, reduced pilocarpine-stimulated salivary secretion and decreased salivary α-amylase activity were observed in rats with DM2. However, the exact cause of diabetic microangiopathy in the salivary glands remains unclear.

The present study is the first to comprehensively assess salivary inflammatory and anti-inflammatory profiles in the salivary glands of rats with DM2. Significantly increased concentrations of pro-inflammatory cytokines (IL-1α, TNF-α, IL-6, IL-7, IL-18), anti-inflammatory cytokines (IL-10), Th1 (IL-12), Th17 (IL-17), chemokines (MCP-lα/CCL2, MIP-1α/CCL3, RANTES/CCL5,

MIP-3α/CCL20, GRO-1α/CXCL1), growth factors (M-CSF, GM-CSF, VEGF), and pro-inflammatory enzymes (MPO, GLU) were observed in rats with DM2. The inflammatory profile of the salivary glands did not indicate dominance of a specific branch of the immune system. However, elevated concentrations of all pro-inflammatory cytokines were detected, with the exception of IL-1β. Moreover, the activity of lysosomal exoglycosidases and MMPs involved in salivary gland remodeling (HEX, HEX B, MMP-2, and MMP-9; with the exception of FUC) was significantly higher in the PGs and SMGs of DM2 rats compared to controls. Two-factor analysis of variance (ANOVA) demonstrated that inflammation and catabolism of salivary glycoconjugates were more pronounced in the PGs of rats with DM2. Increasing levels of inflammatory biomarkers were associated with declining salivary secretory function, suggesting a direct contribution of inflammation to salivary dysfunction.

Salivary cytokines, chemokines and growth factors

Systemic inflammation associated with chronic hyperglycemia appears to be a major source of oral inflammation in patients with DM2.[58] Neutrophil granulocytes (PMNs) activated under these conditions adhere to the vascular endothelium (adhesion) and/or each other (aggregation), initiating an inflammatory response.[59,60] Additionally, the endocrine activity in adipose tissue interferes with the expression of insulin receptors and contributes to complications in distant organs.[59] In DM2, activation of the κB kinase/NF-κB (IKK/NF-κB and c-Jun N-terminal kinase (JNK1)) pathways reduces the sensitivity of target tissues to insulin and stimulates excessive cytokine and chemokine production.[61,62] Increased oxidative stress and carbonyl stress also promote oral inflammation.[63] Glucose autoxidation, increased metabolism of the polyol and sorbitol pathways and, above all, non-enzymatic protein glycation are responsible for the overproduction of ROS, which, by positive feedback, increase the production of cytokines, chemokines and growth factors.[64,65] Advanced glycation end products (AGEs) formed by non-enzymatic coupling of glucose to the amino groups of proteins appear to play a crucial role in promoting oral inflammation.[66] Our previous study demonstrated AGE aggregation and accumulation in salivary glands of obese rats under hyperglycemic conditions, resulting in reduced unstimulated and stimulated salivary secretion.[32] Binding of AGEs to the receptor for advanced glycation end products (RAGE) activates the NF-κB pro-inflammatory pathway and increases the sensitivity of PMNs and monocytes to stimuli that induce the production of inflammatory mediators.[67] The activation of RAGE also increases NADPH oxidase (NOX) activity, the most important pro-oxidant enzyme of the oral cavity, which further enhances inflammation.[68,69]

Neutrophil granulocyte-produced cytokines increase the expression of receptors for intercellular adhesion molecules mediated by neutrophil adhesion to the vascular endothelium and PMN passage into tissues.[70] Migration of inflammatory cells toward the chemotactic stimulus requires cooperation with MMPs, particularly type IV collagenases. These are capable of degrading the ECM and basement membranes of blood vessels.[71–73] Therefore, increased activity of MMP-2 and MMP-9 in the salivary glands of rats with DM2 is expected. Under these conditions, bioavailability of cytotoxic nitric oxide (NO) increases alongside endothelin I and angiotensin II production. Released cytokines and chemokines further enhance vascular permeability, impair fibrinolysis and promote leukocyte adhesion to endothelial cells by enhancing the expression of adhesin.[74,75] Vascular wall remodeling that occurs in the target tissues leads to a loss of elasticity and flexibility, impairs microcirculatory blood flow and induces hypoxia in the surrounding tissues.[76–78] Notably, VEGF levels were markedly increased in the PGs and SMGs of DM2 rats. As a major angiogenic factor, VEGF enhances vascular permeability.[79,80] These findings are supported by elevated salivary HEX activity, a sensitive and early indicator of diabetic microangiopathy, endothelial dysfunction and organ damage in the vascular area.[81,82]

Greater inflammatory changes in the PGs may be related to the physiological absence of adipocytes in the SMGs and their exclusive presence in the PGs.[83,84] Indeed, metabolic disturbances in DM2 promote adipocyte hyperplasia and hypertrophy not only in the adipose tissue, but also in distant organs.[85] Chronic hyperglycemia stimulates adipocytes to release MCP-1α, which increases the influx of monocytes into the site of inflammation and promotes their transformation into macrophages.[85,86] Macrophages, through the release of pro-inflammatory cytokines (↑IL-1α, ↑IL-1β, ↑TNF-α, and ↑IL-6), enhance inflammation, which explains the increase in GLU and MPO activities. β-D-glucuronidase is a recognized marker of chronic oral inflammation.[39] Increased levels of GLU are indicative of enhanced neutrophil influx and activity at the site of inflammation, while elevated MPO levels serve as a confirmation of inflammation in the salivary glands. This enzyme is released by neutrophils and monocytes during the activation of inflammatory cells. Myeloperoxidase also exacerbates oxidative stress, leading to tissue damage, as well as initiates and sustains the inflammatory process. Therefore, MPO has been identified as a marker linking inflammation and oxidative stress.[87]

Salivary vs. serum inflammatory profiles

Statistically significant changes in the serum contents of pro-inflammatory cytokines (↑IL-1α, ↑IL-1β, ↑TNF-α, ↑IL-6), anti-inflammatory cytokines (↓IL-10), Th1 (↑INF-γ, ↑IL-2, ↑IL-12), Th-17 (↓IL-17), chemokines

(↑RANTES/CCL5), and growth factors (↑VEGF) were noted in DM2 rats compared to controls. Since salivary and serum biomarker levels were generally not correlated, local inflammatory changes appear to differ from systemic inflammation. Indeed, most cytokines act in a paracrine and autocrine manner, and salivary cytokines are mainly derived from inflammatory cells infiltrating salivary glands.[23,88] This hypothesis is supported by the negative correlations between salivary secretion, α-amylase activity and the content of cytokines, chemokines and growth factors exclusively in the salivary glands of rats with DM2. Also, multivariate linear regression analysis further confirmed that salivary inflammatory biomarkers increase with a decrease in salivary secretory function. Although our experiment is observational in nature, salivary gland dysfunction in DM2 may increase with the progression of inflammation. Previous studies have shown that IL-1α, IL-1β and TNF-α inhibit acetylcholine release from parasympathetic endings of salivary glands,[89] weakening the glandular cell response and reducing saliva production in patients with Sjögren's syndrome.[90,91]

In contrast to salivary glands, reduced contents of anti-inflammatory cytokines (IL-10) and Th17 (IL-17) were observed in serum of DM2 rats. The decrease in IL-17 levels in diabetic rats is particularly puzzling. Although IL-17 is mainly known for its pro-inflammatory properties, this molecule also exhibits potent anti-inflammatory effects.[92] Indeed, IL-17 plays an important role in protection against extracellular pathogens and may stimulate an inflammatory/anti-inflammatory response in autoimmune diseases.[93] Further research is necessary to elucidate the role of IL-17 in autoimmune diseases, including DM2.

Salivary exoglycosidases, matrix metalloproteinases and apoptosis

Extracellular matrix proteins play a key role in salivary gland function by providing a reservoir of water and electrolytes and by participating in the differentiation of salivary cells and the exchange of nutrients and ions between them.[94] The main components of the oral ECM are glycoconjugates (i.e., glycoproteins, proteoglycans and sphingolipids), which undergo continuous remodeling. Lysosomal exoglycosidases are responsible for replacing old ECM components with newly synthesized structures.[39] In the present study, a significant increase in the activity of salivary exoglycosidases (HEX and HEX B) was observed in both PGs and SMGs of diabetic rats. HEX is the most active enzyme among salivary exoglycosidases.[39,40] Elevated activity of HEX and HEX B indicates increased degradation of oral-forming glycoconjugates and acquired membrane glycoproteins, which leads to disruption of oral homeostasis. The sugar chains of glycoconjugates are involved in the majority of oral processes, including interactions between glycoproteins and bacteria present in saliva.[39,40] These compounds contribute to pH regulation, mucosal hydration, immune defense, and maintenance of ecological balance in the oral cavity.[95] Increased activity of lysosomal exoglycosidases (HEX, HEX B) and MMPs (MMP-2, MMP-9) in the salivary glands of rats with DM2 promotes increased degradation of ECM components. This process may disturb communication between salivary cells and nerve endings, inhibit neural transmission and signal generation, and ultimately reduce protein and water secretion.[35,40,96,97] Additionally, the increased activity of salivary exoglycosidases/MMPs may have accelerated the degradation of salivary gland glycoconjugates, which was not followed by the renewal of ECM components (↓FUC in the PGs and SMGs in diabetic rats). This imbalance may reduce the secretory active area of the glands and limit protein synthesis. Similar changes have been reported in type 1 diabetes mellitus.[35] Chronic inflammation is likely the cause of increased salivary gland remodeling in DM2. Previous studies have shown that IL-1α, IL-1β, TNF-α, and IL-6 increase the activity of MMPs and activate intracellular apoptosis pathways, thereby contributing to salivary hypofunction.[98–100] Consistent with these observations, we found significantly increased MMP-2, MMP-9 and caspase-3 activities in the PGs and SMGs of DM2 rats.

Limitations

Due to limited study material, we were unable to perform histological and histochemical analyses, which represents an important limitation. However, numerous reports describing degenerative and hypertrophic changes in salivary glands of diabetic subjects are available in the literature.[56,101,102] It would also have been valuable to assess systemic inflammatory markers 72 h after DM2 induction. However, owing to ethical restrictions, only minimal blood sampling was permitted to confirm disease development. Further studies on the role of inflammation in the pathogenesis of salivary gland dysfunction in DM2 are required, in particular, molecular analyses determining the role of specific signaling pathways in the induction and promotion of inflammation. A detailed analysis of salivary ECM remodeling, including the activity and expression of other MMPs and tissue inhibitors of metalloproteinases (TIMPs), is also warranted. Finally, this study was conducted using a standard animal model of DM2 involving male Wistar rats. Future research should address salivary gland inflammation in both men and women with diabetes.

Conclusions

Type 2 diabetes mellitus is associated with an increased production of cytokines, chemokines and growth factors at both systemic (serum) and local (salivary gland) levels.

The parotid gland appears to be the main source of salivary cytokines, chemokines and growth factors. Elevated salivary biomarkers of inflammation are accompanied by reduced secretory activity of the salivary glands, which may suggest a direct contribution of inflammation to salivary gland dysfunction. In DM2, increased ECM remodeling and increased apoptosis of glandular tissue are observed, with more pronounced impairment in the PGs. Further in vitro and in vivo studies are required to fully assess the role of inflammation in diabetic salivary gland hypofunction.

Ethics approval and consent to participate

The study was approved by the Local Ethical Committee for Animal Experiments in Olsztyn, Poland (approval No. 62/2021).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Guthrie RA, Guthrie DW. Pathophysiology of diabetes mellitus. *Crit Care Nurs Q*. 2004;27(2):113–125. doi:10.1097/00002727-200404000-00003
2. Jacobs AM. Diabetes mellitus. *Clin Podiatr Med Surg*. 1993;10(2):231–248. PMID:8481881.
3. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2011;34 Suppl 1(Suppl 1):S62–S69. doi:10.2337/dc11-S062
4. Wu Y, Ding Y, Tanaka Y, Zhang W. Risk factors contributing to type 2 diabetes and recent advances in the treatment and prevention. *Int J Med Sci*. 2014;11(11):1185–1200. doi:10.7150/ijms.10001
5. Kolb H, Mandrup-Poulsen T. The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. *Diabetologia*. 2010;53(1):10–20. doi:10.1007/s00125-009-1573-7
6. Gerich JE. Contributions of insulin-resistance and insulin-secretory defects to the pathogenesis of type 2 diabetes mellitus. *Mayo Clin Proc*. 2003;78(4):447–456. doi:10.4065/78.4.447
7. Campbell IW. Epidemiology and clinical presentation of type 2 diabetes. *Value Health*. 2000;3 Suppl 1:3–6. doi:10.1046/j.1524-4733.2000.36014.x
8. Donath MY. Targeting inflammation in the treatment of type 2 diabetes. *Diabetes Obes Metab*. 2013;15 Suppl 3:193–196. doi:10.1111/dom.12172
9. Lamey PJ, Darwazeh AM, Frier BM. Oral disorders associated with diabetes mellitus. *Diabet Med*. 1992;9(5):410–416. doi:10.1111/j.1464-5491.1992.tb01809.x
10. Leite RS, Marlow NM, Fernandes JK, Hermayer K. Oral health and type 2 diabetes. *Am J Med Sci*. 2013;345(4):271–273. doi:10.1097/MAJ.0b013e31828bdedf
11. López-Pintor RM, Casañas E, González-Serrano J, et al. Xerostomia, hyposalivation, and salivary flow in diabetes patients. *J Diabetes Res*. 2016;2016:4372852. doi:10.1155/2016/4372852
12. Mata AD, Marques D, Rocha S, et al. Effects of diabetes mellitus on salivary secretion and its composition in the human. *Mol Cell Biochem*. 2004;261(1–2):137–142. doi:10.1023/b:mcbi.0000028748.40917.6f
13. Soysa NS, Samaranayake LP, Ellepola ANB. Diabetes mellitus as a contributory factor in oral candidosis. *Diabet Med*. 2006;23(5):455–459. doi:10.1111/j.1464-5491.2005.01701.x
14. Borgnakke WS, Anderson PF, Shannon C, Jivanescu A. Is there a relationship between oral health and diabetic neuropathy? *Curr Diab Rep*. 2015;15(11):93. doi:10.1007/s11892-015-0673-7
15. Proctor GB. The physiology of salivary secretion. *Periodontol 2000*. 2016;70(1):11–25. doi:10.1111/prd.12116
16. Zalewska A, Maciejczyk M, Szulimowska J, Imierska M, Błachnio-Zabielska A. High-fat diet affects ceramide content, disturbs mitochondrial redox balance, and induces apoptosis in the submandibular glands of mice. *Biomolecules*. 2019;9(12):877. doi:10.3390/biom9120877
17. Zalewska A, Ziembicka D, Żendzian-Piotrowska M, Maciejczyk M. The impact of high-fat diet on mitochondrial function, free radical production, and nitrosative stress in the salivary glands of Wistar rats. *Oxid Med Cell Longev*. 2019;2019:2606120. doi:10.1155/2019/2606120
18. Kołodziej U, Maciejczyk M, Miąsko A, et al. Oxidative modification in the salivary glands of high fat-diet induced insulin resistant rats. *Front Physiol*. 2017;8:20. doi:10.3389/fphys.2017.00020
19. Maciejczyk M, Matczuk J, Żendzian-Piotrowska M, et al. Eight-week consumption of high-sucrose diet has a pro-oxidant effect and alters the function of the salivary glands of rats. *Nutrients*. 2018;10(10):1530. doi:10.3390/nu10101530
20. Squadrito G, Cucinotta D. The late complications of diabetes mellitus. *Ann Ital Med Int*. 1991;6(1 Pt 2):126–136. PMID:1742148.
21. Avogaro A, Fadini GP. Microvascular complications in diabetes: A growing concern for cardiologists. *Int J Cardiol*. 2019;291:29–35. doi:10.1016/j.ijcard.2019.02.030
22. Jonsson R, Brokstad KA, Jonsson MV, Delaleu N, Skarstein K. Current concepts on Sjögren's syndrome – classification criteria and biomarkers. *Eur J Oral Sci*. 2018;126 Suppl 1(Suppl 1):37–48. doi:10.1111/eos.12536
23. Klimiuk A, Zalewska A, Knapp M, Skutnik-Radziszewska A, Maciejczyk M. Could inflammation contribute to salivary gland dysfunction in patients with chronic heart failure? *Front Immunol*. 2022;13:1005981. doi:10.3389/fimmu.2022.1005981
24. Morawska K, Maciejczyk M, Zięba S, et al. Cytokine/chemokine/growth factor profiles contribute to understanding the pathogenesis of the salivary gland dysfunction in euthyroid Hashimoto's thyroiditis patients. *Mediators Inflamm*. 2021;2021:3192409. doi:10.1155/2021/3192409
25. Skutnik-Radziszewska A, Maciejczyk M, Flisiak I, et al. Enhanced inflammation and nitrosative stress in the saliva and plasma of patients with plaque psoriasis. *J Clin Med*. 2020;9(3):745. doi:10.3390/jcm9030745
26. Maciejczyk M, Pietrzykowska A, Zalewska A, Knaś M, Daniszewska I. The significance of matrix metalloproteinases in oral diseases. *Adv Clin Exp Med*. 2016;25(2):383–390. doi:10.17219/acem/30428
27. Furman BL. Streptozotocin-induced diabetic models in mice and rats. *Curr Protoc*. 2021;1(4):e78. doi:10.1002/cpz1.78

28. Yu S, Cheng Y, Zhang L, et al. Treatment with adipose tissue-derived mesenchymal stem cells exerts anti-diabetic effects, improves long-term complications, and attenuates inflammation in type 2 diabetic rats. *Stem Cell Res Ther*. 2019;10(1):333. doi:10.1186/s13287-019-1474-8
29. Zalewska A, Knaś M, Zendzian-Piotrowska M, et al. Antioxidant profile of salivary glands in high fat diet-induced insulin resistance rats. *Oral Dis*. 2014;20(6):560–566. doi:10.1111/odi.12173
30. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacol Res*. 2005;52(4):313–320. doi:10.1016/j.phrs.2005.05.004
31. Glaeser JD, Ju D, Tawackoli W, et al. Advanced glycation end product inhibitor pyridoxamine attenuates IVD degeneration in type 2 diabetic rats. *Int J Mol Sci*. 2020;21(24):9709. doi:10.3390/ijms21249709
32. Zalewska A, Kuć J, Zięba S, et al. N-acetylcysteine supplementation did not reverse mitochondrial oxidative stress, apoptosis, and inflammation in the salivary glands of hyperglycemic rats. *Nutr Diabetes*. 2021;11(1):35. doi:10.1038/s41387-021-00177-w
33. Zalewska A, Szarmach I, Żendzian-Piotrowska M, Maciejczyk M. The effect of N-acetylcysteine on respiratory enzymes, ADP/ATP ratio, glutathione metabolism, and nitrosative stress in the salivary gland mitochondria of insulin resistant rats. *Nutrients*. 2020;12(2):458. doi:10.3390/nu12020458
34. Fisher E, Stain E. Alpha amylase from human saliva. *Biochem Prep*. 1961:27–33.
35. Maciejczyk M, Kossakowska A, Szulimowska J, et al. Lysosomal exoglycosidase profile and secretory function in the salivary glands of rats with streptozotocin-induced diabetes. *J Diabetes Res*. 2017;2017:9850398. doi:10.1155/2017/9850398
36. Kruidenier L, Kuiper I, Van Duijn W, et al. Imbalanced secondary mucosal antioxidant response in inflammatory bowel disease. *J Pathol*. 2003;201(1):17–27. doi:10.1002/path.1408
37. Troeberg L, Nagase H. Monitoring metalloproteinase activity using synthetic fluorogenic substrates. *Curr Protoc Protein Sci*. 2004;Chapter 21:21.16.1–21.16.9. doi:10.1002/0471140864.ps2116s33
38. Meki ARMA, Esmail EEDF, Hussein AA, Hassanein HM. Caspase-3 and heat shock protein-70 in rat liver treated with aflatoxin B1: Effect of melatonin. *Toxicon*. 2004;43(1):93–100. doi:10.1016/j.toxicon.2003.10.026
39. Chojnowska S, Zalewska A, Knaś M, et al. Determination of lysosomal exoglycosidases in human saliva. *Acta Biochim Pol*. 2014;61(1):85–90. PMID:24644545.
40. Zalewska A, Knaś M, Niczyporuk M, et al. Salivary lysosomal exoglycosidases profiles in patients with insulin-dependent and noninsulin-dependent diabetes mellitus. *Adv Clin Exp Med*. 2013;22(5):659–666. PMID:24285450.
41. Swarnakar S, Mishra A, Chaudhuri SR. The gelatinases and their inhibitors: The structure–activity relationships. *Exp Suppl*. 2012;103:57–82. PMID:22642190.
42. Asadi M, Taghizadeh S, Kaviani E, et al. Caspase-3: Structure, function, and biotechnological aspects. *Biotechnol Appl Biochem*. 2022;69(4):1633–1645. doi:10.1002/bab.2233
43. Grisi DC, Vieira IV, de Almeida Lima AK, et al. The complex interrelationship between diabetes mellitus, oral diseases and general health. *Curr Diabetes Rev*. 2022;18(3):e220321192408. doi:10.2174/1573399817666210322153210
44. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. *J Oral Pathol Med*. 2018;47(5):526–530. doi:10.1111/jop.12712
45. Kudiyirickal MG, Pappachan JM. Diabetes mellitus and oral health. *Endocrine*. 2015;49(1):27–34. doi:10.1007/s12020-014-0496-3
46. Sreebny LM, Yu A, Green A, Valdini A. Xerostomia in diabetes mellitus. *Diabetes Care*. 1992;15(7):900–904. doi:10.2337/diacare.15.7.900
47. Zachariasen RD. Diabetes mellitus and xerostomia. *Compendium*. 1992;13(4):314,316,318–322. PMID:1521277.
48. Atkinson JC, Grisius M, Massey W. Salivary hypofunction and xerostomia: Diagnosis and treatment. *Dent Clin North Am*. 2005;49(2):309–326. doi:10.1016/j.cden.2004.10.002
49. Pankhurst CL, Smith EC, Rogers JO, Dunne SM, Jackson SH, Proctor G. Diagnosis and management of the dry mouth: Part 1. *Dent Update*. 1996;23(2):56–62. PMID:8948194.
50. Saito I. Pathology of salivary gland dysfunction and restoration of function. *Pathol Int*. 2021;71(5):304–315. doi:10.1111/pin.13079
51. Su H, Velly AM, Salah MH, et al. Altered redox homeostasis in human diabetes saliva. *J Oral Pathol Med*. 2012;41(3):235–241. doi:10.1111/j.1600-0714.2011.01092.x
52. Ryo K, Yamada H, Nakagawa Y, et al. Possible involvement of oxidative stress in salivary gland of patients with Sjogren's syndrome. *Pathobiology*. 2006;73(5):252–260. doi:10.1159/000098211
53. Ninche N, Kwak M, Ghazizadeh S. Diverse epithelial cell populations contribute to the regeneration of secretory units in injured salivary glands. *Development*. 2020;147(19):dev192807. doi:10.1242/dev.192807
54. Silvers AR, Som PM. Salivary glands. *Radiol Clin North Am*. 1998;36(5):941–966. doi:10.1016/s0033-8389(05)70070-1
55. El Sadik A, Mohamed E, El Zainy A. Postnatal changes in the development of rat submandibular glands in offspring of diabetic mothers: Biochemical, histological and ultrastructural study. *PLoS One*. 2018;13(10):e0205372. doi:10.1371/journal.pone.0205372
56. Huang Y, Mao QY, Shi XJ, et al. Disruption of tight junctions contributes to hyposalivation of salivary glands in a mouse model of type 2 diabetes. *J Anat*. 2020;237(3):556–567. doi:10.1111/joa.13203
57. Carda C, Mosquera-Lloreda N, Salom L, Gomez de Ferraris ME, Peydró A. Structural and functional salivary disorders in type 2 diabetic patients. *Med Oral Patol Oral Cir Bucal*. 2006;11(4):E309–E314. PMID:16816810.
58. Graves DT, Liu R, Alikhani M, Al-Mashat H, Trackman PC. Diabetes-enhanced inflammation and apoptosis – impact on periodontal pathology. *J Dent Res*. 2006;85(1):15–21. doi:10.1177/154405910608500103
59. Lontchi-Yimagou E, Sobngwi E, Matsha TE, Kengne AP. Diabetes mellitus and inflammation. *Curr Diab Rep*. 2013;13(3):435–444. doi:10.1007/s11892-013-0375-y
60. van den Oever IAM, Raterman HG, Nurmohamed MT, Simsek S. Endothelial dysfunction, inflammation, and apoptosis in diabetes mellitus. *Mediators Inflamm*. 2010;2010:792393. doi:10.1155/2010/792393
61. Donath MY, Böni-Schnetzler M, Ellingsgaard H, Halban PA, Ehses JA. Cytokine production by islets in health and diabetes: Cellular origin, regulation and function. *Trends Endocrinol Metab*. 2010;21(5):261–267. doi:10.1016/j.tem.2009.12.010
62. Ehses JA, Böni-Schnetzler M, Faulenbach M, Donath MY. Macrophages, cytokines and beta-cell death in type 2 diabetes. *Biochem Soc Trans*. 2008;36(Pt 3):340–342. doi:10.1042/BST0360340
63. Kesarwala AH, Krishna MC, Mitchell JB. Oxidative stress in oral diseases. *Oral Dis*. 2016;22(1):9–18. doi:10.1111/odi.12300
64. Darenskaya MA, Kolesnikova LI, Kolesnikov SI. Oxidative stress: Pathogenetic role in diabetes mellitus and its complications and therapeutic approaches to correction. *Bull Exp Biol Med*. 2021;171(2):179–189. doi:10.1007/s10517-021-05191-7
65. Rendra E, Riabov V, Mossel DM, Sevastyanova T, Harmsen MC, Kzhyshkowska J. Reactive oxygen species (ROS) in macrophage activation and function in diabetes. *Immunobiology*. 2019;224(2):242–253. doi:10.1016/j.imbio.2018.11.010
66. Byun K, Yoo Y, Son M, et al. Advanced glycation end-products produced systemically and by macrophages: A common contributor to inflammation and degenerative diseases. *Pharmacol Ther*. 2017;177:44–55. doi:10.1016/j.pharmthera.2017.02.030
67. Hou J, Zheng D, Fung G, et al. Mangiferin suppressed advanced glycation end products (AGEs) through NF-κB deactivation and displayed anti-inflammatory effects in streptozotocin and high fat diet-diabetic cardiomyopathy rats. *Can J Physiol Pharmacol*. 2016;94(3):332–340. doi:10.1139/cjpp-2015-0073
68. Tóbon-Velasco JC, Cuevas E, Torres-Ramos MA. Receptor for AGEs (RAGE) as mediator of NF-kB pathway activation in neuroinflammation and oxidative stress. *CNS Neurol Disord Drug Targets*. 2014;13(9):1615–1626. doi:10.2174/18715273136661408061448 31

69. Hudson BI, Lippman ME. Targeting RAGE signaling in inflammatory disease. *Annu Rev Med*. 2018;69:349–364. doi:10.1146/annurev-med-041316-085215
70. Neumann M, Kownatzki E. The effect of adherence on the generation of reactive oxygen species by human neutrophilic granulocytes. *Agents Actions*. 1989;26(1–2):183–185. doi:10.1007/BF02126602
71. Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK. Extracellular matrix structure. *Adv Drug Deliv Rev*. 2016;97:4–27. doi:10.1016/j.addr.2015.11.001
72. Laeeq S, Faust R. Modeling the cholesteatoma microenvironment: Coculture of HaCaT keratinocytes with WS1 fibroblasts induces MMP-2 activation, invasive phenotype, and proteolysis of the extracellular matrix. *Laryngoscope*. 2007;117(2):313–318. doi:10.1097/01.mlg.0000251164.26405.1a
73. Kisling A, Lust RM, Katwa LC. What is the role of peptide fragments of collagen I and IV in health and disease? *Life Sci*. 2019;228:30–34. doi:10.1016/j.lfs.2019.04.042
74. Cheng ZJ, Vapaatalo H, Mervaala E. Angiotensin II and vascular inflammation. *Med Sci Monit*. 2005;11(6):RA194–RA205. PMID:15917731.
75. Banes-Berceli AKL, Ketsawatsomkron P, Ogbi S, Patel B, Pollock DM, Marrero MB. Angiotensin II and endothelin-1 augment the vascular complications of diabetes via JAK2 activation. *Am J Physiol Heart Circ Physiol*. 2007;293(2):H1291–H1299. doi:10.1152/ajpheart.00181.2007
76. Spinetti G, Kraenkel N, Emanueli C, Madeddu P. Diabetes and vessel wall remodelling: From mechanistic insights to regenerative therapies. *Cardiovasc Res*. 2008;78(2):265–273. doi:10.1093/cvr/cvn039
77. Lunder M, Janić M, Šabovič M. Prevention of vascular complications in diabetes mellitus patients: Focus on the arterial wall. *Curr Vasc Pharmacol*. 2019;17(1):6–15. doi:10.2174/1570161116666180206113755
78. Hu CT, Shao YD, Liu YZ, et al. Oxidative stress in vascular calcification. *Clin Chim Acta*. 2021;519:101–110. doi:10.1016/j.cca.2021.04.012
79. Melincovici CS, Boşca AB, Şuşman S, et al. Vascular endothelial growth factor (VEGF) – key factor in normal and pathological angiogenesis. *Rom J Morphol Embryol*. 2018;59(2):455–467. PMID:30173249.
80. Nardi GM, Ferrara E, Converti I, et al. Does diabetes induce the vascular endothelial growth factor (VEGF) expression in periodontal tissues? A systematic review. *Int J Environ Res Public Health*. 2020;17(8):2765. doi:10.3390/ijerph17082765
81. Watts GF, Vlitos MA, Morris RW, Price RG. Urinary N-acetyl-beta-D-glucosaminidase excretion in insulin-dependent diabetes mellitus: Relation to microalbuminuria, retinopathy and glycaemic control. *Diabete Metab*. 1988;14(5):653–658. PMID:3234588.
82. Skrha J, Hilgertová J. Relationship of serum N-acetyl-beta-glucosaminidase activity to oxidative stress in diabetes mellitus. *Clin Chim Acta*. 1999;282(1–2):167–174. doi:10.1016/s0009-8981(99)00025-x
83. Skarstein K, Aqrawi LA, Øijordsbakken G, Jonsson R, Jensen JL. Adipose tissue is prominent in salivary glands of Sjögren's syndrome patients and appears to influence the microenvironment in these organs. *Autoimmunity*. 2016;49(5):338–346. doi:10.1080/08916934.2016.1183656
84. Nam K, Maruyama CL, Trump BG, et al. Post-irradiated human submandibular glands display high collagen deposition, disorganized cell junctions, and an increased number of adipocytes. *J Histochem Cytochem*. 2016;64(6):343–352. doi:10.1369/0022155416646089
85. Lin Y, Berg AH, Iyengar P, et al. The hyperglycemia-induced inflammatory response in adipocytes: The role of reactive oxygen species. *J Biol Chem*. 2005;280(6):4617–4626. doi:10.1074/jbc.M411863200
86. Tomás E, Lin YS, Dagher Z, et al. Hyperglycemia and insulin resistance: Possible mechanisms. *Ann N Y Acad Sci*. 2002;967:43–51. doi:10.1111/j.1749-6632.2002.tb04262.x
87. Aratani Y. Myeloperoxidase: Its role for host defense, inflammation, and neutrophil function. *Arch Biochem Biophys*. 2018;640:47–52. doi:10.1016/j.abb.2018.01.004
88. Tan Z, Wang L, Li X. Composition and regulation of the immune microenvironment of salivary gland in Sjögren's syndrome. *Front Immunol*. 2022;13:967304. doi:10.3389/fimmu.2022.967304
89. Tzioufas AG, Tsonis J, Moutsopoulos HM. Neuroendocrine dysfunction in Sjogren's syndrome. *Neuroimmunomodulation*. 2008;15(1):37–45. doi:10.1159/000135622
90. Jonsson R. Disease mechanisms in Sjögren's syndrome: What do we know? *Scand J Immunol*. 2022;95(3):e13145. doi:10.1111/sji.13145
91. Marshall LL, Stevens GA. Management of primary Sjögren's syndrome. *Consult Pharm*. 2018;33(12):691–701. doi:10.4140/TCP.n.2018.691
92. Xiao E, Mattos M, Vieira GHA, et al. Diabetes enhances IL-17 expression and alters the oral microbiome to increase its pathogenicity. *Cell Host Microbe*. 2017;22(1):120–128.e4. doi:10.1016/j.chom.2017.06.014
93. Akiyama S, Sakuraba A. Distinct roles of interleukin-17 and T helper 17 cells among autoimmune diseases. *J Transl Autoimmun*. 2021;4:100104. doi:10.1016/j.jtauto.2021.100104
94. Hannas AR, Pereira JC, Granjeiro JM, Tjäderhane L. The role of matrix metalloproteinases in the oral environment. *Acta Odontol Scand*. 2007;65(1):1–13. doi:10.1080/00016350600963640
95. Shetty PK, Pattabiraman TN. Salivary proteolytic activities in periodontitis, gingivitis and diabetes mellitus. *Indian J Clin Biochem*. 1998;13(1):46–51. doi:10.1007/BF02873443
96. Nater UM, Rohleder N. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology*. 2009;34(4):486–496. doi:10.1016/j.psyneuen.2009.01.014
97. Pinkstaff CA. Salivary glands, glycoconjugates and diabetes mellitus. *Eur J Morphol*. 1996;34(3):187–190. doi:10.1076/ejom.34.3.187.13039
98. Cabral-Pacheco GA, Garza-Veloz I, Castruita-De la Rosa C, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. *Int J Mol Sci*. 2020;21(24):9739. doi:10.3390/ijms21249739
99. Zhang X, Chen W, De Paiva CS, et al. Desiccating stress induces CD4+ T-cell-mediated Sjögren's syndrome-like corneal epithelial apoptosis via activation of the extrinsic apoptotic pathway by interferon-γ. *Am J Pathol*. 2011;179(4):1807–1814. doi:10.1016/j.ajpath.2011.06.030
100. Kimura-Shimmyo A, Kashiwamura SI, Ueda H, et al. Cytokine-induced injury of the lacrimal and salivary glands. *J Immunother*. 2002;25 Suppl 1:S42–S51. doi:10.1097/00002371-200203001-00007
101. Alqahtani MS, Hassan SS. Immunohistochemical evaluation of the pathological effects of diabetes mellitus on the major salivary glands of albino rats. *Eur J Dent*. 2022;17(2):485–491. doi:10.1055/s-0042-1749159
102. Lilliu MA, Solinas P, Cossu M, et al. Diabetes causes morphological changes in human submandibular gland: A morphometric study. *J Oral Pathol Med*. 2015;44(4):291–295. doi:10.1111/jop.12238

Original paper

Prediction of non-coding RNAs in *Fusobacterium nucleatum*-infected mice using machine learning

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Abstract

Background. The anaerobic commensal *Fusobacterium nucleatum* is scarce in healthy subgingival dental biofilms but is highly prevalent in periodontal pockets. Numerous genome-wide association studies and gene expression studies using microarrays or RNA sequencing (RNA-Seq) have been performed to better understand the genetic architecture of periodontal disease. However, these investigations have limited predictive capacity for identifying RNAs, particularly non-coding RNAs (ncRNAs). The mechanism of regulation of ncRNAs by *F. nucleatum* to alter disease progression in mice has not been thoroughly investigated.

Objectives. The aim of the study was to predict previously uncharacterized ncRNAs in *F. nucleatum*-infected mice using machine learning (ML).

Material and methods. Long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) were identified from the periodontitis gene expression dataset (GSE225589) obtained from the Gene Expression Omnibus (GEO) database and subsequently preprocessed. Long non-coding RNAs and circRNAs were labeled based on the gene expression. Transcriptomic features were analyzed using 3 ML algorithms: random forest (RF); adaptive boosting (AdaBoost); and naïve Bayes (NB). The dataset was labeled and divided into training (80%) and testing (20%) subsets with cross-validation. Additionally, receiver operating characteristic (ROC) curves, confusion matrices and area under the ROC curve (AUC) values were determined.

Results. The RF and AdaBoost models outperformed the NB model in classifying lncRNAs and circRNAs. Both RF and AdaBoost achieved an AUC of 100%, whereas the NB model achieved a slightly lower AUC of 92%.

Conclusions. This study is the first to apply ML to predict ncRNAs in *F. nucleatum*-infected mice using transcriptomic data. Random forest and AdaBoost showed superior classification performance in identifying lncRNAs and circRNAs associated with the infection. Further studies with larger cohorts and external validation are needed to confirm these findings.

Keywords: periodontal disease, machine learning, transcriptomics, non-coding RNAs

Highlights

- Machine learning enabled the identification of lncRNAs and circRNAs associated with *Fusobacterium nucleatum* infection in mice, marking the first application of machine learning in this context.
- Random forest and AdaBoost achieved perfect classification performance (AUC = 1.000), outperforming naïve Bayes and demonstrating the robustness of ensemble learning approaches for ncRNA prediction.
- The identified ncRNAs demonstrate strong potential as diagnostic or prognostic biomarkers; however, larger datasets and external validation are needed to confirm their clinical applicability.

Introduction

Chronic multifactorial inflammatory periodontitis progressively destroys the tooth-supporting structures and is associated with dysbiotic dental plaque biofilms.[1,2] Approximately 100 different microbial species inhabit the human oral cavity.[3,4] Although most oral bacteria are commensals, a small proportion are hazardous.[5,6] The formation of bacterial biofilms on tooth surfaces is identified as the main cause of periodontal disease.[7–11] Common oral bacteria include *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, *Treponema* species, and *Eubacterium* species.

Oral bacteria produce various polysaccharides and glycoproteins that enable co-aggregation with planktonic microorganisms and adhesion to surfaces (co-adhesion). The acquired pellicle formed on tooth surfaces, composed primarily of salivary glycoproteins and antibodies, facilitates bacterial attachment. Biofilm-associated microorganisms exhibit distinct characteristics compared with planktonic bacteria, including close spatial organization, production of a self-generated extracellular matrix, reduced metabolic activity, and quorum sensing mechanisms that enhance coordinated survival and persistence.

The most prevalent colonizers are gram-positive facultative anaerobes, particularly *Streptococcus* and *Actinomyces* species.[12] The build-up of dental plaque causes a decrease in oxygen levels, favoring colonization by anaerobic bacteria. *Fusobacterium* species serve as bridging organisms between primary and secondary colonizers.[13] Socransky et al. classified the microorganisms into microbial complexes based on their color.[14] The red and orange complexes are strongly associated with periodontal disease in the subgingival region.[7,15] Among these organisms, *F. nucleatum* is considered a key species in the etiology of periodontitis.

Fusobacterium nucleatum is an anaerobic commensal bacterium present in low concentrations in healthy subgingival biofilms[16] but enriched in periodontal pockets.[17] It serves as an important bridging organism between early colonizers and periodontal pathogens.[18] *Fusobacterium nucleatum* has been also associated with colorectal cancer, ulcerative colitis, cardiovascular disease,[19,20] and extraoral infections that might be dangerous in pregnancy,[19] supporting its classification as an opportunistic pathogen.

Experimental periodontitis models in rats have demonstrated that *F. nucleatum* infection can cause abscess formation and alveolar bone loss.[21] Infection with *F. nucleatum* and *A. actinomycetemcomitans* stimulates the production of cytokines such as interleukin (IL)-1. Additionally, tumor necrosis factor (TNF) and IL-17 synergize with IL-1 to enhance the synthesis and expression of additional cytokines (e.g., IL-6), defensins and endothelial activation markers, thereby amplifying the immune response.

Recently discovered non-coding RNA (ncRNAs) include long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs).[22,23] Many diseases are initiated and progress through cis- and trans-regulatory gene expression mechanisms.[24–26] LncRNAs affect the gene regulatory network of host–pathogen interactions and influence biological processes such as cell proliferation, motility, and immune or inflammatory response in cardiovascular, inflammatory and autoimmune diseases.[27–29] The biological functions of circRNAs are not yet fully understood. According to previous studies, circRNAs may function as microRNA (miRNA) sponges, thereby inhibiting miRNA activity, increasing the expression of miRNA target genes, modulating cytokine expression, promoting cell cycle progression, and inhibiting apoptosis.[30–32] Both lncRNAs and circRNAs have been identified as potential diagnostic markers.[33,34]

However, in vitro and in vivo studies have investigated *F. nucleatum* invasion and host response mechanisms.[35–39] Previous research has shown that *F. nucleatum* infection activates the inflammatory response, induces cytokine production and sends danger signals to human glomerular endothelial cells (GECs).[37,40] The molecular mechanisms underlying immune responses to *F. nucleatum* infection in mice are unknown. To understand the genetic architecture of this complex trait, several genome-wide association studies and gene expression investigations using microarrays or RNA sequencing (RNA-Seq) have

been conducted. The outcomes of these investigations, however, do not provide robust insights. Li and Liang developed LncDC, a Python-based tool that outperformed 6 other algorithms in classifying lncRNAs and mRNAs.[41] Using osteosarcoma transcriptomic data, they identified 97 novel lncRNAs, providing potential diagnostic biomarkers or therapy targets.[41] However, no studies have investigated how *F. nucleatum* regulates ncRNAs to influence disease progression in mice. The application of alternative methodologies can improve the generalizability of the results. Machine learning (ML) techniques, combined with resampling strategies, offer a powerful framework for identifying ncRNAs in mice infected with *F. nucleatum*.

Due to their computational effectiveness in identifying generalizable patterns from high-dimensional datasets generated from small samples, ML techniques have been utilized to analyze high-throughput deep sequencing data.[42] Therefore, the current study aimed to apply ML to predict previously uncharacterized ncRNAs in mice infected with *F. nucleatum*.

Material and methods

Source and processing of data

The gene expression dataset for periodontitis (GSE225589) was retrieved from the Gene Expression Omnibus (GEO) database. A comprehensive search of microarray studies was conducted using the keywords "periodontitis" and "*Rattus norvegicus*". The epitranscriptomic gene expression data was exported to Microsoft Excel (Microsoft Corp., Redmond, USA), and outliers were removed. The dataset was preprocessed to identify lncRNAs and circRNAs, which were then classified and labeled based on their gene expression features. The processed data was subjected to exploratory data analysis.

The Orange Data Mining tool (https://orangedataminingcom/download) was used for gene expression analysis, including data upload, transformation, user-interactive visualization, as well as model inference and visualization. These stages involve data input and transformation, interactive visualization, drawing conclusions about data models, and model representation. Data is typically received, processed and visualized by a workflow component, which then generates the analysis for further processing (Fig. 1,2).

Random forest (RF) parameters included up to 100 decision trees, with no strict limitation on tree depth and feature selection. Adaptive boosting (AdaBoost) parameters included the number of boosting iterations and learning rate with several estimators set to 50, and the SAMME (Stagewise Additive Modeling using a Multi-class Exponential loss) learning algorithm. Data balancing techniques, such as oversampling, undersampling and

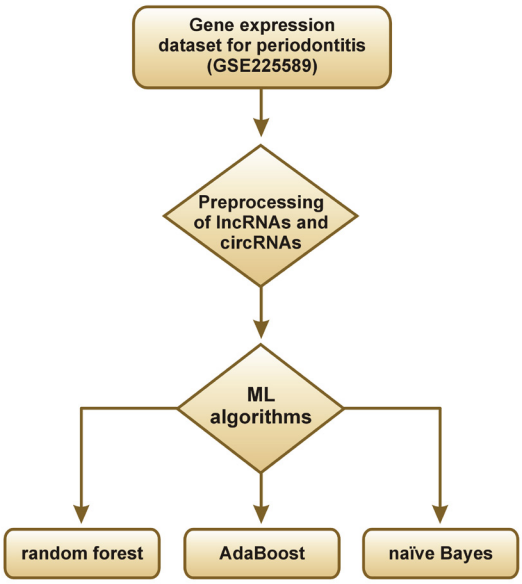

Fig. 1. Flowchart of research methodology
ML – machine learning; AdaBoost – adaptive boosting.

class weighting, were used to address class imbalances in the dataset. Model performance was evaluated using a confusion matrix, receiver operating characteristic (ROC) curve, and area under the ROC curve (AUC). The AUC provides an aggregate performance measure across different classification thresholds, while the confusion matrix summarizes information about the model's performance in each class. The ROC curve provides a trade-off between sensitivity and specificity, thus facilitating the selection of an appropriate threshold.

Genes with constant expression patterns within the same class were assigned greater weight than genes with erratic expression. This method reduces non-informative genes to improve classification accuracy.

Adaptive boosting

Adaptive boosting is an ensemble learning algorithm that combines weak learners to build a strong classifier. The algorithm fits a classifier to the original dataset and then trains additional classifiers on weighted versions of the data. In each iteration, the weights of misclassified instances are increased, allowing the model to focus on more challenging cases. AdaBoost can be applied to gene expression data from DNA microarray and RNA-Seq platforms for classification tasks.

Random forest

Random forest is a sophisticated ML method used for classification and regression. It constructs numerous decision trees during the training phase. The output of this process is the class, that is, the mode of the classes (classification) or the mean prediction (regression) of the individual trees. Random forest can predict clinical

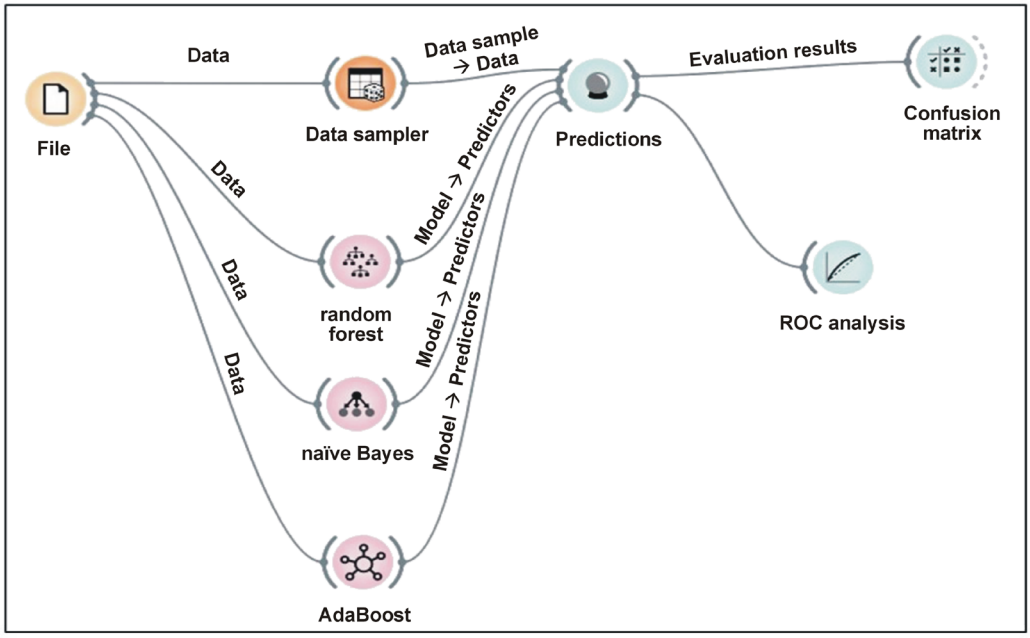

Fig. 2. Orange machine learning (ML) framework
ROC – receiver operating characteristic.

outcomes in gene expression (genes) using profile datasets with several characteristics. The prediction task is a classification problem involving feature representations. The primary objective of this approach is to achieve high classification accuracy. Random forest is effective for the analysis of gene expression datasets, due to its capacity to manage many input variables and their complex interactions. High-dimensional data, such as gene expression profiles, is prone to overfitting; however, this method is robust.

Naïve Bayes

Naïve Bayes (NB) is a basic yet successful ML method that applies Bayes' theorem with strong (naïve) independence assumptions among features. Due to its simplicity and efficiency, NB is frequently applied to high-dimensional datasets, including gene expression data.

The naïve Bayes classifier (NBC) is widely used in pattern recognition tasks involving gene expression. However, classical estimations of location and scale parameters are sensitive to outliers, which complicates the interpretation of gene expression data using the classical NBC.

Results

The predictive performance of lncRNA and circRNA disease associations was evaluated using stratified 20-fold cross-validation. In each iteration, the dataset was partitioned into training and testing subsets, with most samples used for training and the remainder for testing. During each cycle, similarities between lncRNAs and circRNAs were recalculated based solely on known training associations.

After estimating the association probabilities of the test samples, the samples were arranged according to their association scores. Samples with higher scores were considered more likely to represent true lncRNA–circRNA disease associations. A sample was deemed positive if an observed association existed in the lncRNA–cirRNA disease node pair and its association score exceeded a predefined threshold. The true positive rate (TPR) and the false positive rate (FPR) were calculated as follows (Equation 1 and Equation 2):

$$TPR = TP/(TP + FN) \quad (1)$$

$$FPR = FP/(FP + TN) \quad (2)$$

where:
FN – number of false negatives (incorrectly classified positive samples);
FP – number of false positives (incorrectly classified negative samples);
TP – number of true positives (correctly classified positive samples);
TN – number of true negatives (correctly classified negative samples).

By varying the threshold, ROC curves were generated. The AUC provided an overall measure of the predictive capability of the model.[43]

Due to a considerable imbalance between observed lncRNA–disease associations (positive samples) and unobserved associations (negative samples), precision–recall (PR) curves and the area under the PR curve (AUPR)

were used to assess predictive performance.[44] Precision and recall were defined as follows (Equations 3 and 4):

$$\text{Precision} = TP/(TP + FP) \quad (3)$$

$$\text{Recall} = TP/(TP + FN) \quad (4)$$

A precision of 1 indicates that FP = 0. Similarly, a recall of 1 indicates that FN = 0. An ideal classifier achieves precision and recall of 1, corresponding to 0 false positives and false negatives. As the number of false negatives increases, the recall decreases because the denominator (TP + FN) grows relative to TP.

To jointly evaluate precision and recall, the F1 score was calculated (Equation 5):

$$\text{F1 score} = \frac{\text{precision} \times \text{recall}}{\text{precision} + \text{recall}} \times 2 \quad (5)$$

The AUC–ROC curves and, most importantly, recall, precision, specificity, and accuracy, were derived from the confusion matrix. The target feature in this test consisted of 2 groups of predictors: lncRNAs and circRNAs. In this study, stratified 20-fold cross-validation was applied. The confusion matrix summarizes the number of true positives, true negatives, false positives, and false negatives generated by the model on the test data.

Identification and prediction of non-coding RNAs and construction of classification models

Twenty-fold cross-validation was used to determine model accuracy and AUC after classification using the ML framework (Fig. 2). The outcomes demonstrated that, for lncRNAs and circRNAs, accuracy and AUC value reached 100%. To classify lncRNAs and circRNAs, RF, AdaBoost and NB classification models were built. The RF and AdaBoost models achieved the highest accuracy (AUC = 1.000), whereas the NB model achieved a slightly lower AUC of 0.926 (Table 1). These findings indicate that ML-based classification algorithms targeting lncRNAs and circRNAs demonstrate high diagnostic accuracy.

Prediction and expression of non-coding RNAs

The ROC analysis was conducted to assess the predictive utility of the selected lncRNAs and circRNAs. All targeted ncRNAs demonstrated AUC values greater than 0.7, indicating acceptable predictive performance. These findings suggest that the identified ncRNAs may serve as prognostic markers in mice infected with *F. nucleatum*. However, the individual AUCs of the selected lncRNAs and circRNAs were lower than the overall classification performance of the RF, AdaBoost and NB models, suggesting that the integrated ML approach provides superior predictive accuracy compared with single biomarkers.

Evaluation of the results using the confusion matrix of the RF model correctly classified 110 lncRNAs as true positives as 114 circRNAs as true negatives (Table 2).

Similar to the RF model, AdaBoost correctly identified 110 lncRNAs as true positives and 114 circRNAs as true negatives (Table 3).

The NB model correctly classified 98 lncRNAs as true positives and 98 circRNAs as true negatives. However, it misclassified 12 lncRNAs as false negatives and 16 circRNAs as false positives (Table 4).

Table 2. Confusion matrix of the random forest (RF) model

Non-coding RNAs		Actual		
		lncRNAs	circRNAs	∑
Predicted	lncRNAs	110	0	110
	circRNAs	0	114	114
	∑	110	114	224

The table presents the predicted and actual classification labels generated by the model. LncRNAs and circRNAs represent the 2 predicted classes. The data is presented as the number of samples in each category. The bottom row and the rightmost column indicate the total number of samples per class and the overall sum of samples, respectively.

Table 3. Confusion matrix of the adaptive boosting (AdaBoost) model

Non-coding RNAs		Actual		
		lncRNAs	circRNAs	∑
Predicted	lncRNAs	110	0	110
	circRNAs	0	114	114
	∑	110	114	224

The table presents the predicted and actual classification labels generated by the model. LncRNAs and circRNAs represent the 2 predicted classes. The data is presented as the number of samples in each category. The bottom row and the rightmost column indicate the total number of samples per class and the overall sum of samples, respectively.

Table 1. Performance metrics of the machine learning (ML) algorithms used for classifying long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs)

Model	AUC	CA	F1 score	Precision	Recall	MCC
Random forest	1.000	1.000	1.000	1.000	1.000	1.000
AdaBoost	1.000	1.000	1.000	1.000	1.000	1.000
Naïve Bayes	0.926	0.875	0.875	0.876	0.875	0.751

AdaBoost – adaptive boosting; AUC – area under the receiver operating characteristic (ROC) curve; CA – classification accuracy; MCC – Matthews correlation coefficient.

Table 4. Confusion matrix of the naïve Bayes (NB) model

Non-coding RNAs		Actual		
		lncRNAs	circRNAs	∑
Predicted	lncRNAs	98	12	110
	circRNAs	16	98	114
	∑	110	114	224

The table presents the predicted and actual classification labels generated by the model. LncRNAs and circRNAs represent the 2 predicted classes. The data is presented as the number of samples in each category. The bottom row and the rightmost column indicate the total number of samples per class and the overall sum of samples, respectively.

Discussion

Fusobacterium nucleatum is a gram-negative, obligate anaerobic bacillus named for its slender, spindle-shaped morphology.[45] It has been associated with the etiology of periodontitis and contributes to dental plaque formation.[46] Acting as a bridging organism between commensal bacteria and periodontal pathogens on tooth and epithelial surfaces, *F. nucleatum* plays a crucial role in mediating physical interactions between gram-positive and gram-negative bacteria.[47] The bacterium expresses multiple adhesins that facilitate binding to other microorganisms and cells, thereby enhancing pathogenicity. The most important virulence factor in *F. nucleatum* is *Fusobacterium* adhesin A (FadA), an adhesion protein.[19] *Fusobacterium* adhesin A exists as mature FadA (mFadA), a secreted protein of 111 amino acids, and pre-FadA, a 129-amino-acid precursor form.[48] The active complex (FadAc), composed of both forms, enables binding and invasion of host cells.[48,49]

Machine learning applications in transcriptomics have quickly expanded, enabling computational analysis of gene expression data generated by techniques such as RNA-Seq. Using ML approaches, researchers can detect differentially expressed genes, categorize samples into groups, predict gene functions, and find hidden molecular patterns. These capabilities provide novel biological insights and support biomarker discovery. A variety of ML methods, including decision trees, support vector machines, RFs, and deep learning models, have been applied to address the high dimensionality and complexity of transcriptomic datasets. Such strategies have great potential to contribute to our understanding of disease diagnosis, gene regulation, and the development of personalized medicine.[50] Because experimental biological research is often time-consuming and costly, computational prediction of disease-associated lncRNAs via bioinformatics has become increasingly common. In recent years, many lncRNA–disease association prediction (LDAP) models have been proposed, including models based on biological networks, models independent of known lncRNA–disease relationships, and ML-based frameworks.

Non-coding RNAs are RNA molecules that do not encode proteins but play significant regulatory functions in several biological processes. Once considered transcriptional noise, ncRNAs are now recognized due to their numerous applications and influence on gene expression.

Well-established subclasses of RNAs include transfer RNAs (tRNAs), which are essential for protein synthesis by transporting amino acids to the ribosomes, and ribosomal RNAs (rRNAs), which form the structural core of ribosomes involved in protein synthesis. Extensive research has been conducted on these RNAs due to their pivotal role in the fundamental functioning of cells. In addition, messenger RNAs (mRNAs) can bind to short RNA molecules, called miRNAs, which can either promote or hinder the translation of mRNAs. MicroRNAs regulate gene expression and play a role in a variety of cellular functions, such as differentiation, development and disease progression.

Another well-known family of ncRNAs is lncRNAs. These larger RNA molecules are transcribed from the genome, yet they do not encode proteins. LncRNAs are involved in several regulatory processes, including post-transcriptional processing, chromatin remodeling and transcriptional control. Additionally, they have been linked to crucial biological processes such as cellular differentiation, the etiology of disease and embryonic development.

Recent studies have also highlighted the functions of enhancer RNAs (eRNAs) and circRNAs in gene regulation. CircRNAs are covalently closed RNA molecules produced during transcription by a back-splicing process. These cells can affect the expression of genes through interactions with RNA-binding proteins or by acting as miRNA sponges. While eRNAs are expected to facilitate enhancer–promoter interactions and modulate gene transcription, they are translated from enhancer regions of the genome.

The functional roles and regulatory mechanisms of ncRNAs are the subject of ongoing research. Dysregulation of ncRNAs has been associated with numerous diseases, including cancer, neurological disorders, periodontal disease, and cardiovascular conditions. The potential of ncRNAs in diagnostics and therapeutics could be utilized in the development of novel biomarkers and focused therapies.

In the present study, we aimed to predict ncRNAs associated with *F. nucleatum* infection in mice to better understand the etiology of *F. nucleatum*-related disorders. Orange, an open-source data visualization and ML toolkit developed in Python, has been used to build and analyze predictive models. The tool supports popular algorithms like RF, neural networks, AdaBoost, NB, and logistic regression, but in our study, 3 models were used: RF; AdaBoost; and NB.

The gene expression dataset for periodontitis (GSE225589) was obtained from the GEO database.

Using GEO2R, 250 differentially expressed genes (DEGs) were identified by comparison with appropriate controls. The 3 ML algorithms, namely RF, AdaBoost and NB, were evaluated for accuracy. The RF and AdaBoost models achieved superior and consistent accuracy compared with the NB model. Specifically, RF achieved an AUC of 1.000, classification accuracy (CA) of 1.000, F1 score of 1.000, precision of 1.000, recall of 1.000, and Matthews correlation coefficient (MCC) of 1.000. AdaBoost demonstrated identical performance metrics. In contrast, the NB model achieved an AUC value of 0.926, CA of 0.875, F1 score of 0.875, precision of 0.876, recall of 0.875, and MCC of 0.751.

Integrating proteomics or metabolomics data could further enhance understanding of disease mechanisms and improve predictive performance. Machine learning-based identification of indicators or pathways of periodontitis may facilitate the development of targeted therapeutic strategies or diagnostic tools.[51]

Several limitations of the present study should be acknowledged, including the relatively small sample size, reliance on a single dataset, and the lack of external validation. The identified DEGs and predictive models require replication in independent datasets to confirm accuracy. Understanding the biological significance and mechanisms of these genes is crucial for further insights. Future research should incorporate multi-omics integration, longitudinal analyses, functional experiments, assessment of potential confounding factors, and development of clinically applicable predictive models. These approaches could provide a more comprehensive understanding of the molecular mechanisms underlying periodontitis, identify novel biomarkers, examine gene expression changes over time, validate the biological relevance of DEGs, and improve patient management and outcomes.

A previous study investigating oral colonization of mice with *P. gingivalis*, *Treponema denticola* and *T. forsythia* demonstrated enhanced intrabony defects and alveolar bone resorption (ABR) during polymicrobial infection.[47] These pathogens successfully established oral colonization and induced ABR. Another research demonstrated that chronic oral infection with *F. nucleatum* has been shown to induce symptoms of periodontal disease in mice, spread via hematogenous routes, alter the host immune system and periodontal risk factors, and cause both pro- and anti-inflammatory reactions.[49] According to earlier in vitro studies, the significantly elevated levels of immunoglobulin G (IgG) and IgM after chronic infection suggest that *F. nucleatum* functions as a powerful B-cell mitogen.[52] Additionally, the mitogenic activity has been attributed to the Toll-like receptor 2 (TLR2) adjuvant outer membrane porin FomA.[53]

The endogenous retroviral-associated adenocarcinoma lncRNA (EVADR) and keratin-7 antisense RNA (KRT7-AS) were among 43 upregulated lncRNAs identified in a recent transcriptome investigation of *F. nucleatum*-infected colon cancer cells.[54] *Fusobacterium nucleatum* has been reported to promote the progression of oral squamous cell carcinoma (OSCC).[54] A lncRNA, MIR4435-2HG-5p, has been shown to be upregulated in *F. nucleatum*-infected OSCC cells, where it functions as a miRNA-296-5p sponge, activates AKT2 signaling, and contributes to AKT2-induced carcinogenesis.[55–59]

The role of lncRNAs in *F. nucleatum*-infected mice sheds light on the complex regulatory mechanisms underlying diseases associated with this pathogen. Previous studies suggest that *F. nucleatum* infection can alter host lncRNA expression, which may be crucial in regulating host immune responses, promoting bacterial survival and accelerating disease progression. One of the main functions of lncRNAs in infected mice is the control of host immune responses. LncRNAs can function as molecular scaffolds or ploys, interacting with proteins or other RNA molecules to regulate signaling pathways and gene expression involved in immune control. Additionally, they can control the expression of pro- and anti-inflammatory genes, ultimately affecting the host's ability to mount an effective immune response against *F. nucleatum*.

A recent study used mono- and dinucleotide sequences to predict ncRNA regulatory functions.[60] A back propagation (BP) neural network with principal component analysis and the Levenberg–Marquardt algorithm trained ncRNAs with accuracies of 81.3% for mixed bacterial ncRNAs and 93.3% for prokaryotic tRNAs.[60] Another study trained a classifier (MncR) using RNAcentral data and reported over 97% accuracy in classifying ncRNA classes.[61] More recently, ML methods such as logistic regression, RF, eXtreme Gradient Boosting (XGBoost), and decision trees have been employed to distinguish coding from non-coding transcripts and classify ncRNAs. In human datasets, RF achieved accuracies exceeding 83%. Our study obtained similar and highly accurate results.[51]

Additionally, lncRNAs may influence cellular functions such as cell division, apoptosis and epithelial–mesenchymal transition, all of which are significant in *F. nucleatum*-associated disorders. Dysregulation of specific lncRNAs in infected mice may promote aberrant cell behavior, potentially contributing to tumor growth, invasion or metastasis in malignancies associated with *F. nucleatum*. Furthermore, lncRNAs may participate in the direct communication between host cells and *F. nucleatum*. According to recent research, bacterial lncRNAs may be transferred to host cells, where they can modulate the expression of host genes and signaling pathways. These lncRNAs may play a role in the establishment and persistence of *F. nucleatum* infection by interfering with host cellular functions.

To fully elucidate the predicted roles of lncRNAs in *F. nucleatum*-infected mice, further studies using experimental and computational methods are necessary. Integrating transcriptomic analysis with functional assays,

including knockdown or overexpression studies, will help clarify the involvement of particular lncRNAs to immune modulation, bacterial persistence and disease development. Additionally, integrative methodologies, such as network analysis and ML, can facilitate the identification of key lncRNA–mRNA interaction networks and improve understanding of the complex regulatory networks.

Investigating lncRNA-mediated regulatory mechanisms in *F. nucleatum* infection enhances our understanding of host–pathogen interactions and identifies potential therapeutic targets aimed at regulating immune responses, limiting bacterial survival or delaying the onset of disease in *F. nucleatum*-related conditions.

Conclusions

This study represents the first application of ML approaches to predict ncRNAs in a mouse model infected with *F. nucleatum*. The superior performance of the RF and AdaBoost models highlights the robustness of ensemble learning for transcriptomic classification. The identified lncRNAs and circRNAs may serve as promising candidates for biomarker development and for advancing our understanding of host–pathogen regulatory mechanisms. Nevertheless, validation in larger and independent datasets, along with functional experimental studies, is essential to confirm their biological and clinical relevance.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Clin Periodontol*. 2018;45 Suppl 20:S162–S170. doi:10.1111/jcpe.12946
2. Shrivastava D, Natoli V, Srivastava KC, et al. Novel approach to dental biofilm management through guided biofilm therapy (GBT): A review. *Microorganisms*. 2021;9(9):1966. doi:10.3390/microorganisms9091966
3. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol*. 2005;43(11):5721–5732. doi:10.1128/JCM.43.11.5721-5732.2005
4. Shrivastava D, Srivastava KC, Dayakara JK, et al. Bactericidal activity of crevicular polymorphonuclear neutrophils in chronic periodontitis patients and healthy subjects under the influence of areca nut extract: An in vitro study. *Appl Sci*. 2020;10(14):5008. doi:10.3390/app10145008
5. Hajishengallis G, Darveau RP, Curtis MA. The keystone-pathogen hypothesis. *Nat Rev Microbiol*. 2012;10(10):717–725. doi:10.1038/nrmicro2873
6. Bibi T, Khurshid Z, Rehman A, Imran E, Srivastava KC, Shrivastava D. Gingival crevicular fluid (GCF): A diagnostic tool for the detection of periodontal health and diseases. *Molecules*. 2021;26(5):1208. doi:10.3390/molecules26051208
7. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL. Microbial complexes in subgingival plaque. *J Clin Periodontol*. 1998;25(2):134–144. doi:10.1111/j.1600-051x.1998.tb02419.x
8. Darveau RP. Periodontitis: A polymicrobial disruption of host homeostasis. *Nat Rev Microbiol*. 2010;8(7):481–490. doi:10.1038/nrmicro2337
9. Nimbulkar G, Garacha V, Shetty V, et al. Microbiological and clinical evaluation of Neem gel and chlorhexidine gel on dental plaque and gingivitis in 20–30 years old adults: A randomized parallel-armed, double-blinded controlled trial. *J Pharm Bioallied Sci*. 2020;12(Suppl 1):S345–S351. doi:10.4103/jpbs.JPBS_101_20
10. Shrivastava D, Srivastava KC, Ganji KK, Alam MK, Al Zoubi I, Sghaireen MG. Quantitative assessment of gingival inflammation in patients undergoing nonsurgical periodontal therapy using photometric CIELab analysis. *Biomed Res Int*. 2021;2021:1–8. doi:10.1155/2021/6615603
11. Alam MK, Kamal MA, Srivastava KC. Special issue on current concepts and challenges in oral health: Implications for the global population. *Appl Sci*. 2023;13(5):3140. doi:10.3390/app13053140
12. Marsh PD. Dental plaque as a biofilm and a microbial community – implications for health and disease. *BMC Oral Health*. 2006;6 Suppl 1(Suppl 1):S14. doi:10.1186/1472-6831-6-S1-S14
13. Jenkinson HF, Lamont RJ. Oral microbial communities in sickness and in health. *Trends Microbiol*. 2005;13(12):589–595. doi:10.1016/j.tim.2005.09.006
14. Socransky SS, Gibbons RJ, Dale AC, Bortnick L, Rosenthal E, Macdonald JB. The microbiota of the gingival crevice area of man. I. Total microscopic and viable counts and counts of specific organisms. *Arch Oral Biol*. 1963;8:275–280. doi:10.1016/0003-9969(63)90019-0
15. Yadalam PK, Arumuganainar D, Anegundi RV, et al. CRISPR-Cas-based adaptive immunity mediates phage resistance in periodontal red complex pathogens. *Microorganisms*. 2023;11(8):2060. doi:10.3390/microorganisms11082060
16. Caselli E, Fabbri C, D'Accolti M, et al. Defining the oral microbiome by whole-genome sequencing and resistome analysis: The complexity of the healthy picture. *BMC Microbiol*. 2020;20(1):120. doi:10.1186/s12866-020-01801-y
17. Lourenço TGB, Heller D, Silva-Boghossian CM, Cotton SL, Paster BJ, Colombo APV. Microbial signature profiles of periodontally healthy and diseased patients. *J Clin Periodontol*. 2014;41(11):1027–1036. doi:10.1111/jcpe.12302
18. Jung YJ, Jun HK, Choi BK. *Porphyromonas gingivalis* suppresses invasion of *Fusobacterium nucleatum* into gingival epithelial cells. *J Oral Microbiol*. 2017;9(1):1320193. doi:10.1080/20002297.2017.1320193

19. Han YW. *Fusobacterium nucleatum*: A commensal-turned pathogen. *Curr Opin Microbiol*. 2015;23:141–147. doi:10.1016/j.mib.2014.11.013
20. Brennan CA, Garrett WS. *Fusobacterium nucleatum* – symbiont, opportunist and mycobacterium. *Nat Rev Microbiol*. 2019;17(3):156–166. doi:10.1038/s41579-018-0129-6
21. Chaushu S, Wilensky A, Gur C, et al. Direct recognition of *Fusobacterium nucleatum* by the NK cell natural cytotoxicity receptor NKp46 aggravates periodontal disease. *PLoS Pathog*. 2012;8(3):e1002601. doi:10.1371/journal.ppat.1002601
22. Bhan A, Mandal SS. Long noncoding RNAs: Emerging stars in gene regulation, epigenetics and human disease. *ChemMedChem*. 2014;9(9):1932–1956. doi:10.1002/cmdc.201300534
23. Qu S, Yang X, Li X, et al. Circular RNA: A new star of noncoding RNAs. *Cancer Lett*. 2015;365(2):141–148. doi:10.1016/j.canlet.2015.06.003
24. Hirata H, Hinoda Y, Shahryari V, et al. Long noncoding RNA MALAT1 promotes aggressive renal cell carcinoma through Ezh2 and interacts with miR-205. *Cancer Res*. 2015;75(7):1322–1331. doi:10.1158/0008-5472.CAN-14-2931
25. Liu B, Sun L, Liu Q, et al. A cytoplasmic NF-κB interacting long noncoding RNA blocks IκB phosphorylation and suppresses breast cancer metastasis. *Cancer Cell*. 2015;27(3):370–381. doi:10.1016/j.ccell.2015.02.004
26. Yuan JH, Yang F, Wang F, et al. A long noncoding RNA activated by TGF-β promotes the invasion–metastasis cascade in hepatocellular carcinoma. *Cancer Cell*. 2014;25(5):666–681. doi:10.1016/j.ccr.2014.03.010
27. Li Z, Rana TM. Decoding the noncoding: Prospective of lncRNA-mediated innate immune regulation. *RNA Biol*. 2014;11(8):979–985. doi:10.4161/rna.29937
28. Mirza AH, Kaur S, Brorsson CA, Pociot F. Effects of GWAS-associated genetic variants on lncRNAs within IBD and T1D candidate Loci. *PLoS One*. 2014;9(8):e105723. doi:10.1371/journal.pone.0105723
29. Yan B, Yao J, Liu JY, et al. lncRNA-MIAT regulates microvascular dysfunction by functioning as a competing endogenous RNA. *Circ Res*. 2015;116(7):1143–1156. doi:10.1161/CIRCRESAHA.116.305510
30. Deng T, Yang L, Zheng Z, et al. Calcitonin gene-related peptide induces IL-6 expression in RAW264.7 macrophages mediated by mmu_circRNA_007893. *Mol Med Rep*. 2017;16(6):9367–9374. doi:10.3892/mmr.2017.7779
31. Kong Z, Wan X, Zhang Y, et al. Androgen-responsive circular RNA circSMARCA5 is up-regulated and promotes cell proliferation in prostate cancer. *Biochem Biophys Res Commun*. 2017;493(3):1217–1223. doi:10.1016/j.bbrc.2017.07.162
32. Lai Z, Yang Y, Yan Y, et al. Analysis of co-expression networks for circular RNAs and mRNAs reveals that circular RNAs hsa_circ_0047905, hsa_circ_0138960 and has-circRNA7690-15 are candidate oncogenes in gastric cancer. *Cell Cycle*. 2017;16(23):2301–2311. doi:10.1080/15384101.2017.1380135
33. Cui X, Niu W, Kong L, et al. hsa_circRNA_103636: Potential novel diagnostic and therapeutic biomarker in major depressive disorder. *Biomark Med*. 2016;10(9):943–952. doi:10.2217/bmm-2016-0130
34. Zhou M, Zhao H, Wang Z, et al. Identification and validation of potential prognostic lncRNA biomarkers for predicting survival in patients with multiple myeloma. *J Exp Clin Cancer Res*. 2015;34(1):102. doi:10.1186/s13046-015-0219-5
35. Han YW, Shi W, Huang GT, et al. Interactions between periodontal bacteria and human oral epithelial cells: *Fusobacterium nucleatum* adheres to and invades epithelial cells. *Infect Immun*. 2000;68(6):3140–3146. doi:10.1128/IAI.68.6.3140-3146.2000
36. Lee HR, Rhyu IC, Kim HD, et al. In-vivo-induced antigenic determinants of *Fusobacterium nucleatum* subsp. nucleatum. *Mol Oral Microbiol*. 2011;26(2):164–172. doi:10.1111/j.2041-1014.2010.00594.x
37. Bui FQ, Johnson L, Roberts JA, et al. *Fusobacterium nucleatum* infection of gingival epithelial cells leads to NLRP3 inflammasome-dependent secretion of IL-1β and the danger signals ASC and HMGB1. *Cell Microbiol*. 2016;18(7):970–981. doi:10.1111/cmi.12560
38. Kostic AD, Chun E, Robertson L, et al. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe*. 2013;14(2):207–215. doi:10.1016/j.chom.2013.07.007
39. Dharmani P, Strauss J, Ambrose C, Allen-Vercoe E, Chadee K. *Fusobacterium nucleatum* infection of colonic cells stimulates MUC2 mucin and tumor necrosis factor alpha. *Infect Immun*. 2011;79(7):2597–2607. doi:10.1128/IAI.05118-11
40. Hung SC, Huang PR, Almeida-da-Silva CLC, Atanasova KR, Yilmaz O, Ojcius DM. NLRX1 modulates differentially NLRP3 inflammasome activation and NF-κB signaling during *Fusobacterium nucleatum* infection. *Microbes Infect*. 2018;20(9–10):615–625. doi:10.1016/j.micinf.2017.09.014
41. Li M, Liang C. LncDC: A machine learning-based tool for long non-coding RNA detection from RNA-Seq data. *Sci Rep*. 2022;12(1):19083. doi:10.1038/s41598-022-22082-7
42. Sghaireen MG, Al-Smadi Y, Al-Qerem A, et al. Machine learning approach for metabolic syndrome diagnosis using explainable data-augmentation-based classification. *Diagnostics (Basel)*. 2022;12(12):3117. doi:10.3390/diagnostics12123117
43. Hajian-Tilaki K. Receiver operating characteristic (ROC) curve analysis for medical diagnostic test evaluation. *Caspian J Intern Med*. 2013;4(2):627–635. PMID:24009950.
44. Saito T, Rehmsmeier M. The precision-recall plot is more informative than the ROC plot when evaluating binary classifiers on imbalanced datasets. *PLoS One*. 2015;10(3):e0118432. doi:10.1371/journal.pone.0118432
45. Bolstad AI, Jensen HB, Bakken V. Taxonomy, biology, and periodontal aspects of *Fusobacterium nucleatum*. *Clin Microbiol Rev*. 1996;9(1):55–71. doi:10.1128/CMR.9.1.55
46. Patini R, Staderini E, Lajolo C, et al. Relationship between oral microbiota and periodontal disease: A systematic review. *Eur Rev Med Pharmacol Sci*. 2018;22(18):5775–5788. doi:10.26355/eurrev_201809_15903
47. Kolenbrander PE. Oral microbial communities: Biofilms, interactions, and genetic systems. *Annu Rev Microbiol*. 2000;54:413–437. doi:10.1146/annurev.micro.54.1.413
48. Xu M, Yamada M, Li M, Liu H, Chen SG, Han YW. FadA from *Fusobacterium nucleatum* utilizes both secreted and nonsecreted forms for functional oligomerization for attachment and invasion of host cells. *J Biol Chem*. 2007;282(34):25000–25009. doi:10.1074/jbc.M611567200
49. Témoin S, Wu KL, Wu V, Shoham M, Han YW. Signal peptide of FadA adhesin from *Fusobacterium nucleatum* plays a novel structural role by modulating the filament's length and width. *FEBS Lett*. 2012;586(1):1–6. doi:10.1016/j.febslet.2011.10.047
50. Kumar VS, Kumar PR, Yadalam PK, et al. Machine learning in the detection of dental cyst, tumor, and abscess lesions. *BMC Oral Health*. 2023;23(1):833. doi:10.1186/s12903-023-03571-1
51. Tan J, Li X, Zhang L, Du Z. Recent advances in machine learning methods for predicting LncRNA and disease associations. *Front Cell Infect Microbiol*. 2022;12:1071972. doi:10.3389/fcimb.2022.1071972
52. Mangan DF, Lopatin DE. Polyclonal activation of human peripheral blood B lymphocytes by *Fusobacterium nucleatum*. *Infect Immun*. 1983;40(3):1104–1111. doi:10.1128/iai.40.3.1104-1111.1983
53. Toussi DN, Liu X, Massari P. The FomA porin from *Fusobacterium nucleatum* is a Toll-like receptor 2 agonist with immune adjuvant activity. *Clin Vaccine Immunol*. 2012;19(7):1093–1101. doi:10.1128/CVI.00236-12
54. Chen S, Su T, Zhang Y, et al. *Fusobacterium nucleatum* promotes colorectal cancer metastasis by modulating KRT7-AS/KRT7. *Gut Microbes*. 2020;11(3):511–525. doi:10.1080/19490976.2019.1695494
55. Zhang S, Li C, Liu J, et al. *Fusobacterium nucleatum* promotes epithelial–mesenchymal transiton through regulation of the lncRNA MIR4435-2HG/miR-296-5p/Akt2/SNAI1 signaling pathway. *FEBS J*. 2020;287(18):4032–4047. doi:10.1111/febs.15233
56. Özveren N, Sevinç B, Sarıalioğlu Güngör A, Baltacı E, Serindere G, Özgür Ö. Evaluation of knowledge and awareness about teledentistry among dentists and patients living in Turkey. *Dent Med Probl*. 2023;60(4):593–599. doi:10.17219/dmp/150834
57. Motie P, Mohaghegh S, Kouhestani F, Motamedian SR. Effect of mechanical forces on the behavior of osteoblasts: A systematic review of in vitro studies. *Dent Med Probl*. 2023;60(4):673–686. doi:10.17219/dmp/151639

58. Golob Deeb J, Reddy N, Kitten T, Carrico CK, Grzech-Leśniak K. Viability of bacteria associated with root caries after Nd:YAG laser application in combination with various antimicrobial agents: An in vitro study. *Dent Med Probl*. 2023;60(4):649–655. doi:10.17219/dmp/171690
59. Bommala M, Koduganti RR, Panthula VR, et al. Efficacy of root coverage with the use of the conventional versus laser-assisted flap technique with platelet-rich fibrin in class I and class II gingival recession: A randomized clinical trial. *Dent Med Probl*. 2023;60(4):583–592. doi:10.17219/dmp/150887
60. Chantsalnyam T, Siraj A, Tayara H, Chong KT. ncRDense: A novel computational approach for classification of non-coding RNA family by deep learning. *Genomics*. 2021;113(5):3030–3038. doi:10.1016/j.ygeno.2021.07.004
61. Dunkel H, Wehrmann H, Jensen LR, Kuss AW, Simm S. MncR: Late integration machine learning model for classification of ncRNA classes using sequence and structural encoding. *Int J Mol Sci*. 2023;24(10):8884. doi:10.3390/ijms24108884

Original paper

In vitro evaluation of the effect of Er:YAG laser with a fractional PS04 handpiece on microbial biofilm survival

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Abstract

Background. The oral microbiota consists of a diverse range of microorganisms, with *Streptococcus* spp. and *Candida* spp. frequently coexisting in oral infections.

Objectives. The aim of the study was to investigate the impact of Er:YAG (erbium-doped yttrium aluminum garnet) laser therapy, utilizing the PS04 fractional beam, on the in vitro growth and biofilm formation of clinical strains of *Candida albicans*, *Candida glabrata* and *Streptococcus mutans*.

Material and methods. Single- and dual-species planktonic cultures and biofilms were exposed to an Er:YAG laser using a fractional PS04 handpiece. The effects of the laser were evaluated immediately after irradiation and 24 h post-irradiation by measuring colony-forming units per milliliter (CFU/mL). Biofilm biomass (single- and dual-species) was quantified using the crystal violet staining method. The study tested 2 sets of laser parameters: group 1 (T1): 1.5 W, 10 Hz, 30 s, 0.4 J/cm^2, irradiance: 3.9 W/cm^2; and group 2 (T2): 6.15 W, 10 Hz, 30 s, 1.6 J/cm^2, irradiance: 16 W/cm^2. Non-irradiated samples served as controls. The parameters were selected based on their frequent clinical use for snoring treatment and facial rejuvenation.

Results. *Candida albicans* exhibited a significantly greater reduction under T2 settings in comparison to T1 (85.3 ±1.2% vs. 43.9 ±4.5%, respectively; $p = 0.006$) within single-species biofilms. For *C. glabrata*, a significant reduction was observed under T1 parameters (69.8 ±14.9%). Furthermore, *S. mutans* demonstrated a significantly higher reduction at T2 settings (97.1 ±0.6%) compared to T1 settings (81.1 ±19.6%), with statistically significant differences noted between *S. mutans* and both *C. albicans* and *C. glabrata* under T1, as well as between *S. mutans* and *C. glabrata* under T2. In dual-species biofilms, T2 fluence led to greater reductions in *C. glabrata*, *S. mutans* and *C. albicans* in mixed cultures ($p < 0.05$).

Conclusions. The Er:YAG laser, when used in conjunction with the PS04 handpiece, demonstrated a substantial reduction in the biofilms of *C. albicans* and *C. glabrata*. Higher fluence maintained reductions over a 24-h period, particularly in the case of *C. glabrata* and *S. mutans*. This study highlights the antifungal potential of low-fluence laser settings that are commonly used in facial aesthetic procedures and snoring treatment.

Keywords: *Candida albicans*, candidiasis, oral biofilm, erbium laser, *Streptococcus mutans*

Highlights

- The Er:YAG laser with the PS04 fractional handpiece significantly reduced *Candida albicans*, *Candida glabrata* and *Streptococcus mutans* biofilms, with higher fluence showing superior efficacy.
- Higher fluence was more effective against *C. albicans* and *S. mutans*, while lower fluence showed stronger effects on *C. glabrata* over 24 h.
- Mixed-species biofilms responded differently, with *C. glabrata* and *S. mutans* showing greater reductions under higher fluence, supporting tailored laser protocols.
- Low-fluence Er:YAG laser, used in facial aesthetic procedures and snoring treatments, demonstrates antifungal and antibacterial potential in dentistry.

Introduction

The health of the oral cavity is closely interconnected with systemic health.[1–3] Rather than being merely susceptible to microbial colonization, the oral cavity naturally harbors a diverse and dynamic microbial community. This is due to its continuous exposure to the external environment through food, air and other factors, which contribute to the establishment and maintenance of a complex ecosystem of bacteria, fungi and other microorganisms. These microbes can disrupt oral homeostasis, leading to dental caries, periapical infections and candidiasis. In some cases, microbial dysbiosis may contribute to systemic diseases, including cardiovascular disorders, pneumonia and stroke.[4] Among the primary pathogens involved, *Streptococcus mutans* is a key etiological agent in dental caries and a contributor to systemic conditions such as infective endocarditis.[5] Additionally, fungal infections, primarily caused by *Candida albicans*, can disrupt the balance of oral microbiota and pose significant challenges due to their opportunistic nature and resistance.[6]

A critical factor in the development of oral candidiasis is its nature as a multi-species infection, as opposed to being caused by a single fungal species. While *C. albicans* is the predominant species responsible for infections, it exists alongside diverse strains and other *Candida* species, which further complicates infection management. In severe cases, *Candida* spp. may invade the bloodstream, leading to systemic, life-threatening conditions such as candidemia.[6] Conventional treatment for oral candidiasis includes antifungal agents such as nystatin, amphotericin B, or systemic therapies like ketoconazole, fluconazole and 5-fluorocytosine. However, systemic antifungal treatments frequently cause adverse effects, including gastrointestinal disturbances, nephrotoxicity and hepatotoxicity, highlighting the need for innovative strategies.[7] Emerging alternatives, such as probiotics, show promise in preventing oral candidiasis and alleviating symptoms, yet they remain less effective for treating established infections.[8–12]

Laser therapy has emerged as a promising and increasingly popular approach in disease management over the years.[13–25] The antimicrobial action of the laser is particularly noteworthy and widely emphasized.[26–30] Among various types of lasers, the Er:YAG (erbium-doped yttrium aluminum garnet) laser has found wide application in multiple fields of dentistry.[31–36] Studies have demonstrated the effectiveness of the Er:YAG laser in managing microbial biofilm during endodontic treatment[37–41] and reducing biofilm on dental implants affected by peri-implantitis.[42,43] Additionally, Er:YAG laser bleaching has been shown to reduce biofilm formation on enamel surfaces in comparison to conventional bleaching methods without laser application.[44] These findings, along with others, highlight the beneficial effects of Er:YAG laser therapy in inhibiting or reducing biofilm formation in the oral cavity.[45–48] Beyond its efficacy in destroying bacterial biofilms, the Er:YAG laser has also shown potential in reducing *Candida* biofilm formation, further expanding its potential applications in dental and oral health management.[22,49,50]

The present study aims to evaluate the effects of the Er:YAG laser on planktonic single-species, dual-species, and biofilm composed of *C. albicans*, *Candida glabrata* and *S. mutans* strains. Additionally, it explores innovative approaches to eradicate these pathogens using low-fluence laser parameters, specifically designed for the clinical management of snoring and frequently employed in facial aesthetic procedures.

Material and methods

The research was carried out on clinical strains of *C. albicans*, *C. glabrata* and *S. mutans* at the Department of Microbiology, Wroclaw Medical University, Poland, under the approval of the Institutional Research Ethics Committee (approval No. KB-429/2024). A power meter was utilized, and the output was calibrated prior to the commencement of the study.

Culture condition

Clinical cultures of *C. albicans*, *C. glabrata* and *S. mutans* were obtained from patients with candidiasis

and caries lesions and stored at −80°C in Trypticasein Soy Broth (BioMaxima, Lublin, Poland). The strains were frozen in replicates and multiplied before experiments (*S. mutans* on Brain Heart Infusion (BHI) LAB-AGAR™; *Candida* spp. on Sabouraud Dextrose LAB-AGAR™ (BioMaxima)).

Two types of samples were prepared for experimentation:

- planktonic solutions: suspensions of microorganisms (0.5 McFarland standard) were combined with BHI broth (BioMaxima) containing 5% sucrose, forming single-species (*C. albicans*, *C. glabrata*, *S. mutans*) or dual-species combinations (*C. albicans* + *S. mutans*; *C. glabrata* + *S. mutans*; *C. albicans* + *C. glabrata*). The treatments were conducted in dark Eppendorf tubes (SARSTEDT AG & Co. KG, Nümbrecht, Germany);
- biofilm: single- and dual-species biofilms were formed on 96-well polystyrene plates (SPL Life Sciences Co., Ltd., Pocheon, Korea). For single-species biofilms, 100 µL of microbial suspensions (0.5 McFarland standard) and 150 µL of BHI broth were added per well. For dual-species biofilms, 100 µL of each microorganism suspension and 50 µL of BHI broth were used, totaling 250 µL per well. The plates were incubated at 37°C for 24 h under aerobic or CO_2-enhanced conditions (*S. mutans*).

Laser irradiation

The experiment used an Er:YAG laser (LightWalker AT-S; Fotona, Ljubljana, Slovenia) equipped with a fractional PS04 handpiece (Fotona). The laser was operated in a non-contact mode with a 600-µs long pulse duration, maintaining a 10-mm distance from the surface of strain suspension in the Eppendorf tube (plankton) or 96-well polystyrene plates (biofilm) (Fig. 1). The delivery system employed an articulated arm. In both test groups, the same fractional handpiece was used, with 2 variations in parameters. One set was designed for facial rejuvenation, while the other was specifically configured for the clinical treatment of snoring. The control group was biofilm without laser irradiation. The parameters used in the study are outlined in Table 1.

Microorganism quantification

Planktonic cell suspensions

Single- and dual-species planktonic suspensions were exposed to laser light in dark Eppendorf tubes. For the assessment of immediate effects, 100 µL of suspension was serially diluted, and 100 µL from each dilution was plated on BHI agar. The plates were then incubated at 37°C for 24 h under aerobic (yeast) or elevated CO_2 conditions (*S. mutans* and dual-species). Colonies were counted to calculate colony forming units per milliliter (CFU/mL). Control suspensions were subjected to the same protocol without laser exposure.

To assess the effects 24 h post-irradiation, the suspensions underwent incubation at 37°C under aerobic or CO_2 conditions, followed by serial dilution, plating and colony counting. Controls were prepared in a similar manner, without irradiation.

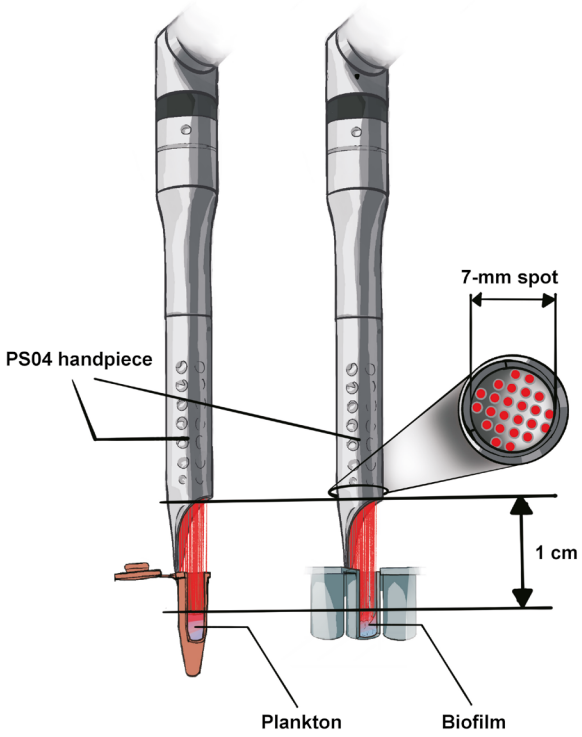

Fig. 1. Methodology of Er:YAG (erbium-doped yttrium aluminum garnet) laser irradiation of planktonic solutions of cells and biofilm

Effect of laser on single- and dual-species biofilm (quantification of CFU/mL)

Biofilm samples cultivated for 24 h were rinsed with 0.9% NaCl to remove any unattached cells. Thereafter, the samples were irradiated with a laser, scraped using sterile swabs and shaken in a 0.5% saponin solution. Subsequently, they were processed via serial dilution and plating.

Table 1. Study groups and laser parameters used during the experiment

Laser	Handpiece	Study group 1 (T1)	Study group 2 (T2)	Control group
Er:YAG	PS04 (fractional)	1.5 W, 10 Hz, 30 s, fluency: 0.4 J/cm^2, LP (600 µs), no tip, 7-mm spot, power density (irradiance): 3.9 W/cm^2	6.15 W, 10 Hz, 30 s, fluency: 1.6 J/cm^2; LP (600 µs), no tip, 7-mm spot, power density (irradiance): 16 W/cm^2	no laser irradiation
		clinical use: facial rejuvenation	clinical use: snoring treatment	

Er:YAG – erbium-doped yttrium aluminum garnet; LP – long pulse.

The plates were incubated at 37°C for 24 h under aerobic or CO_2 conditions, after which CFU/mL values were calculated.

Effect of laser on single- and dual-species biofilm (crystal violet method)

Biofilm samples cultivated for 24 h were rinsed with 0.9% NaCl to remove any unattached cells. Subsequently, the samples were exposed to laser irradiation. Then, they were fixed by drying for 45 min at room temperature. After the drying period, 250 µL of 0.1% crystal violet solution (Chempur®, Piekary Śląskie, Poland) was added to the wells, and the plate was left for 20 min at room temperature. Subsequently, the violet was removed and the wells were washed 3 times in distilled water. Thereafter, they were left to dry for 20 min at room temperature. After adding 200 µL of 95% ethanol (Honeywell, Charlotte, USA), the absorbance was measured at a wavelength (λ) of 540 nm (Asys UVM 340; Biochrom Ltd., Holliston, USA). The control group consisted of a microbial biofilm that had not been exposed to the laser.

Statistical analysis

The Kolmogorov–Smirnov test was performed at a 95% confidence interval (*CI*) to evaluate the normality of data distribution. Differences in microorganism reduction following laser irradiation were analyzed using multivariate analysis of variance (MANOVA). Post-hoc adjustments were implemented using the Holm–Bonferroni method to control for multiple comparisons. All statistical analyses were carried out using Statistica software, v. 12 (StatSoft, Kraków, Poland), with a significance level set at $p < 0.05$.

Results

Reduction of microorganisms in single-species planktonic cultures

The results demonstrate that laser application significantly reduced microorganism cell counts in single-species planktonic cultures in comparison to the control group. The most substantial reductions were observed for *S. mutans* and *C. albicans* when the PS04 handpiece was used directly after irradiation (DAI) at higher fluence settings (T2). Statistically significant differences were identified between the DAI and 24 h after irradiation (24AI) time points for *S. mutans* (80.0 ±1.3% vs. 0.0 ±0.0%; $p < 0.001$) and *C. albicans* (74.0 ±8.3% vs. 31.7 ±10.2%; $p = 0.045$) under T2 settings. Furthermore, higher laser fluence (T2) resulted in a significantly greater reduction of *C. albicans* immediately after irradiation ($p < 0.05$). Conversely, lower fluence (T1) achieved superior reductions for *C. glabrata* and *S. mutans* 24 h post-irradiation compared to T2 ($p < 0.05$) (Table 2).

Reduction of microorganisms in single-species biofilm cultures

The study assessed the reduction of microbial cell counts in biofilm cultures of *C. albicans*, *C. glabrata* and *S. mutans*, directly after irradiation, with an Er:YAG laser used in conjunction with the PS04 handpiece. The results showed that for *C. albicans*, T2 parameters achieved a significantly higher reduction compared to T1 (85.3 ±1.2% vs. 43.9 ±4.5%, respectively; $p = 0.006$), thereby demonstrating enhanced efficacy. In contrast, *C. glabrata* exhibited a substantial reduction under T1 settings, but no reduction at higher fluence (69.8 ±14.9% vs. 0.0 ±0.0%, respectively; $p = 0.022$). *Streptococcus mutans* demonstrated consistently high reductions in both configurations (81.1 ±19.6% for T1 and 97.1 ±0.6% for T2; $p > 0.05$). Inter-group comparisons indicated significant variation among the species ($p < 0.05$), with *S. mutans* showing the highest overall reduction (T2: 97.1 ±0.6%; T1: 81.1 ±19.6%), which was significantly greater compared to *C. albicans* and *C. glabrata* under T1 settings, as well as *C. glabrata* under T2 settings ($p < 0.05$) (Table 3).

Reduction of microorganisms in dual-species planktonic cultures

The results of microbial cell counts in dual-species planktonic cultures (*C. albicans* + *C. glabrata*, *C. albicans* + *S. mutans*, and *C. glabrata* + *S. mutans*) following Er:YAG laser application using the PS04 handpiece revealed significant variability in reduction rates depending on the species combination, laser settings (T1 vs. T2) and timing (DAI vs. 24AI). *Candida albicans* in the *C. albicans* + *S. mutans* mixture showed a significantly higher reduction with T2 settings at 24AI compared to DAI (83.7 ±5.9% vs. 7.4 ±0.1%, respectively; $p = 0.003$). A similar trend was observed for *S. mutans* in the same mixture, which exhibited significant reductions with T1 settings at 24AI compared to DAI (24.9 ±2.3% vs. 0.0 ±0.0%, respectively; $p = 0.004$). For the *C. glabrata* + *S. mutans* biofilm, reductions were significantly greater at 24AI compared to DAI under certain conditions (T2: 39.2 ±10.4% at 24AI vs. 0.0 ±0.0% at DAI; $p = 0.033$). Moreover, a greater microbial reduction for *S. mutans* in the *C. albicans* + *S. mutans* mixture was observed with T2 settings immediately after laser application, while the opposite was observed 24 h post-irradiation ($p < 0.05$). Additionally, for the *C. glabrata* + *S. mutans* mixture, a greater reduction in *S. mutans* was found with T1 settings immediately after laser application compared to results measured 24 h after laser irradiation ($p < 0.05$) (Table 4).

Table 2. Reduction in microbial cell count after laser treatment in single-species planktonic cultures compared to the control group (non-irradiated samples)

Microorganism	Handpiece	Time	Reduction at T1 [%CFU/mL] *M* ±*SD*	Reduction at T2 [%CFU/mL] *M* ±*SD*	*p*-value (T1 vs. T2)
Candida albicans	PS04	DAI	46.5 ±3.0	74.0 ±8.3	0.048*
		24AI	39.2 ±27.4	31.7 ±10.2	0.752
		p-value (DAI vs. 24AI)	0.744	0.045*	–
Candida glabrata		DAI	70.6 ±13.8	65.5 ±5.8	0.680
		24AI	78.8 ±0.6	69.6 ±2.1	0.028*
		p-value (DAI vs. 24AI)	0.489	0.447	–
Streptococcus mutans		DAI	63.3 ±21.7	80.0 ±1.3	0.391
		24AI	39.1 ±7.8	0.0 ±0.0	0.019*
		p-value (DAI vs. 24AI)	0.277	<0.001*	–

* statistically significant ($p < 0.05$, MANOVA); DAI – directly after irradiation; 24AI – 24 h after irradiation; T1 – lower fluence laser settings; T2 – higher fluence laser settings; CFU – colony-forming units; *M* – mean; *SD* – standard deviation.

Table 3. Reduction in microbial cell count directly after laser treatment in single-species biofilm cultures compared to the control group (non-irradiated samples)

Microorganism	Handpiece	Reduction at T1 [%CFU/mL] *M* ±*SD*	Reduction at T2 [%CFU/mL] *M* ±*SD*	*p*-value (T1 vs. T2)
Candida albicans	PS04	43.9 ±4.5	85.3 ±1.2	0.006*
Candida glabrata		69.8 ±14.9	0.0 ±0.0	0.022*
Streptococcus mutans		81.1 ±19.6	97.1 ±0.6	0.366

* statistically significant ($p < 0.05$, MANOVA).

Table 4. Reduction in microbial cell count after laser treatment in dual-species planktonic cultures

Microorganism	Mixture	Handpiece	Time	Reduction at T1 [%CFU/mL] *M* ±*SD*	Reduction at T2 [%CFU/mL] *M* ±*SD*	*p*-value (T1 vs. T2)
Candida albicans (Ca)	Ca+Cg	PS04	DAI	6.7 ±9.4	40.0 ±9.5	0.072
			24AI	42.5 ±14.4	45.9 ±2.1	0.769
			p-value (DAI vs. 24AI)	0.098	0.481	–
	Ca+Sm		DAI	56.9 ±25.5	7.4 ±0.1	0.111
			24AI	67.0 ±3.6	83.7 ±5.9	0.069
			p-value (DAI vs. 24AI)	0.667	0.003*	–
Candida glabrata (Cg)	Ca+Cg	PS04	DAI	24.1 ±3.5	36.9 ±21.6	0.496
			24AI	9.5 ±10.1	7.9 ±11.1	0.894
			p-value (DAI vs. 24AI)	0.193	0.233	–
	Ca+Sm		DAI	39.8 ±13.7	26.3 ±2.0	0.302
			24AI	40.4 ±33.4	4.1 ±5.8	0.270
			p-value (DAI vs. 24AI)	0.985	0.429	–
Streptococcus mutans (Sm)	Ca+Cg	PS04	DAI	0.0 ±0.0	62.7 ±12.8	0.020*
			24AI	24.9 ±2.3	1.4 ±1.9	0.008*
			p-value (DAI vs. 24AI)	0.004*	0.022*	–
	Ca+Sm		DAI	60.0 ±7.1	0.0 ±0.0	0.007*
			24AI	43.8 ±12.8	39.2 ±10.4	0.731
			p-value (DAI vs. 24AI)	0.257	0.033*	–

* statistically significant ($p < 0.05$, MANOVA).

Reduction of microorganisms in dual-species biofilm cultures

Higher laser fluence (T2) exhibited a greater reduction in *C. glabrata* (*C. albicans* + *C. glabrata* and *C. glabrata* + *S. mutans* mixtures), *S. mutans* (*C. albicans* + *S. mutans* and *C. glabrata* + *S. mutans* mixtures) and *C. albicans* (*C. albicans* + *C. glabrata* mixture) ($p < 0.05$). No significant increase in reduction was observed for *C. albicans* in the *C. albicans* + *S. mutans* mixture when comparing the 2 irradiation configurations (Table 5).

Laser-induced biomass reduction in single- and dual-species biofilms evaluated using the crystal violet method

The application of the Er:YAG laser in conjunction with the PS04 handpiece demonstrated varying effectiveness in reducing biofilm biomass, depending on laser power settings. For *C. albicans*, an increase in power from T1 (13.6 ±2.5%) to T2 (14.9 ±1.4%) did not result in a significant reduction ($p = 0.571$). In contrast, *C. glabrata* exhibited a substantial decrease in biomass from T1 to T2 (13.4 ±3.1% vs. 51.15 ±8.0%, respectively; $p = 0.023$), suggesting a greater sensitivity to elevated laser power. *Streptococcus mutans* showed an increase in biomass reduction from T1 (13.8 ±5.3%) to T2 (24.05 ±1.3%), but this change was not statistically significant ($p = 0.099$). Multivariate analysis of variance confirmed significant differences between the species ($p = 0.009$), with the most notable differences observed between *C. glabrata* and *C. albicans* ($p < 0.009$) and between *C. glabrata* and *S. mutans* ($p < 0.022$) (Table 6).

The application of the Er:YAG laser (PS04 handpiece) resulted in varied biomass reduction across different dual-species biofilms, depending on microbial composition and laser power settings. In the *C. albicans* + *S. mutans* biofilm, a substantial biomass reduction was observed, decreasing from T1 to T2 (18.8 ±0.8% vs. 11.4 ±0.9%, respectively; $p = 0.014$), indicating lower efficacy at higher power. Conversely, the *C. glabrata* + *S. mutans* biofilm reduction demonstrated a significant decrease from T1 to T2 (23.2 ±5.4% vs. 0.3 ±0.2%, respectively; $p = 0.027$), suggesting that lower laser power was highly effective against this combination. Moreover, a reduction in biomass was observed for the *C. albicans* + *C. glabrata* biofilm when comparing the T1 and T2 settings (31.0 ±13.2% vs. 49.4 ±14.7%, respectively; $p = 0.318$), implying greater laser efficiency under high-energy conditions (Table 7).

Table 6. Reduction in single-species biofilm biomass after the application of the Er:YAG (erbium-doped yttrium aluminum garnet) laser in conjunction with the crystal violet method

Handpiece	Microorganism	Reduction in biomass [%]		*p*-value
		T1 *M ±SD*	T2 *M ±SD*	
PS04	*Candida albicans* (Ca)	13.6 ±2.5	14.9 ±1.4	0.571
	Candida glabrata (Cg)	13.4 ±3.1	51.2 ±8.0	0.023*
	Streptococcus mutans (Sm)	13.8 ±5.3	24.1 ±1.3	0.099
p-value		0.996[a]	0.009[a] Cg vs. Ca: $p < 0.009$[b] Cg vs. Sm: $p < 0.022$[b]	–

* statistically significant ($p < 0.05$, MANOVA); [a] MANOVA; [b] Tukey's post hoc test.

Table 7. Reduction in dual-species biofilm biomass after the application of the Er:YAG (erbium-doped yttrium aluminum garnet) laser in conjunction with the crystal violet method

Mixture	Reduction in biomass [%]		*p*-value (T1 vs. T2)
	T1 *M ±SD*	T2 *M ±SD*	
Ca+Sm	18.8 ±0.8	11.4 ±0.9	0.014*
Cg+Sm	23.2 ±5.4	0.3 ±0.2	0.027*
Ca+Cg	31.0 ±13.2	49.4 ±14.7	0.318
p-value	0.456[a]	0.021[a] Ca+Cg vs. Ca+Sm: $p = 0.042$[b] Cg+Sm vs. Ca+Cg: $p = 0.021$[b]	–

* statistically significant ($p < 0.05$, MANOVA); [a] MANOVA; [b] Tukey's post hoc test; Ca – *Candida albicans*; Sm – *Streptococcus mutans*; Cg – *Candida glabrata*.

Table 5. Reduction in microbial cell count directly after laser treatment in dual-species biofilm cultures

Microorganism	Mixture	Handpiece	Reduction at T1 [%CFU/mL] *M ±SD*	Reduction at T2 [%CFU/mL] *M ±SD*	*p*-value (T1 vs. T2)
Candida albicans (Ca)	Ca+Cg	PS04	98.1 ±0.6	100.0 ±0.0	0.042*
	Ca+Sm		51.5 ±33.3	98.4 ±0.7	0.184
Candida glabrata (Cg)	Ca+Cg		93.8 ±0.2	99.9 ±0.1	0.001*
	Cg+Sm		61.4 ±2.3	99.2 ±1.1	0.002*
Streptococcus mutans (Sm)	Ca+Sm		61.0 ±10.9	98.6 ±1.1	0.040*
	Cg+Sm		46.9 ±7.8	100.0 ±0.0	0.011*

* statistically significant ($p < 0.05$, MANOVA).

Discussion

The present study is an in-depth examination of the impact of the Er:YAG laser on single-species and dual-species planktonic and biofilm cultures formed by *C. albicans*, *C. glabrata* and *S. mutans*. Additionally, it investigates novel methods for eliminating these pathogens by utilizing low laser parameter settings tailored for the clinical management of facial rejuvenation and snoring. The findings of the current study highlight the potential of the Er:YAG laser with a fractional handpiece as an effective tool for reducing microbial populations in both planktonic and biofilm cultures of *C. albicans*, *C. glabrata* and *S. mutans*. The observed reductions were dependent on laser settings (T1: 0.4 J/cm² vs. T2: 1.6 J/cm²) and microbial species, suggesting that the efficacy of laser treatment can be optimized through the precise customization of parameters to the specific clinical context. Notably, the higher fluence settings (T2) demonstrated superior efficacy in disrupting *C. albicans* and *S. mutans* in single-species and dual-species planktonic cultures immediately after irradiation, while the lower fluence settings (T1) were more effective for *C. glabrata* in single-species planktonic cultures 24 h after irradiation. These results align with earlier studies that emphasize the versatility and efficacy of laser-based approaches in microbial management, particularly in addressing challenges related to biofilm-associated infections in the field of dentistry.[23,48,51]

With respect to the results obtained for planktonic cultures, the application of the Er:YAG laser in conjunction with the PS04 handpiece demonstrated significant reductions in microbial cell counts, particularly for *S. mutans* and *C. albicans*. The most notable reductions were observed under T2 settings, with a 80.0% and 74.0% decrease, respectively, immediately after irradiation. These outcomes are consistent with earlier studies, which also found laser treatment to be effective in reducing the growth of these species, suggesting that the intensity of the laser has a significant impact on microbial survival.[52–54] While *S. mutans* showed no detectable recovery at 24 h post-irradiation, *C. albicans* exhibited partial resurgence, with a reduction of 31.7%. These findings support earlier research indicating that laser efficacy diminishes over time, particularly for fungal organisms like *C. albicans*, which may require repeated interventions for long-term control.[54] Interestingly, while higher fluence (T2) was more effective against *C. albicans* immediately after irradiation, lower fluence (T1) yielded better results for *C. glabrata* and *S. mutans* at 24AI. These results underscore the importance of optimizing laser parameters for different microorganisms, highlighting that microbial responses to laser treatments can vary significantly depending on the organism type and laser fluence.[23,48,51]

In biofilm cultures, the Er:YAG laser, operated with the PS04 handpiece, demonstrated species-specific efficacy in reducing microbial cell counts. This finding is consistent with the existing literature on biofilm management. For single-species biofilms, *S. mutans* exhibited the highest reduction rates, with T2 settings achieving nearly complete eradication (97.1 ±0.6%). This result aligns with the findings reported in the study by Grzech-Leśniak et al., which emphasized the susceptibility of *S. mutans* biofilm to treatments targeting the biofilm matrix using the Nd:YAG (neodymium-doped yttrium aluminum garnet) laser.[23] A comparable response was observed for *C. albicans*, which exhibited a significant reduction under T2 parameters (85.3 ±1.2%), reflecting its vulnerability to interventions that disrupt extracellular polymeric substances (EPS), as discussed by Salehi et al., who highlighted the challenges of disrupting resilient polymicrobial biofilms.[55] In dual-species biofilms, T2 fluence generally achieved greater reductions, particularly for *C. glabrata* and *S. mutans* in mixed cultures, as well as for *C. albicans* in combination with *C. glabrata*. These findings support evidence presented by Salehi et al., which underscored the significance of tailored approaches to address the synergistic interactions between microbial species within biofilms.[55] However, no significant improvement with T2 over T1 settings was observed for *C. albicans* in combination with *S. mutans*, suggesting that interspecies interactions might alter biofilm susceptibility. These findings emphasize the potential of the Er:YAG laser in the management of biofilms and highlight the importance of understanding microbial dynamics to optimize treatment protocols.[48,51,56]

This study builds upon our previous findings, in which the antimicrobial impact of the Nd:YAG laser on *C. albicans* and *S. mutans* was explored using both CFU quantification and Janus green staining.[22] The use of Janus green staining allowed for a detailed assessment of metabolic activity and biofilm viability, providing insights beyond the microbial counts achieved through CFU analysis. This method addressed structural and metabolic biofilm responses, which are critical for understanding the underlying mechanisms of laser-induced microbial reduction. In the current study, CFU quantification was used to evaluate the antimicrobial efficacy of the Er:YAG laser. While this method effectively quantifies microbial reduction, it lacks the ability to visualize biofilm structure and viability. Additionally, the study utilized the Nd:YAG laser for photobiomodulation (PBM) applications,[22,23] targeting deeper tissue interactions. In contrast, the present study explores the surface-level effects of the Er:YAG laser using the fractional PS04 handpiece. The utilization of distinct laser systems and their intended applications highlights the adaptability of these technologies for specific clinical scenarios. While the Nd:YAG laser is effective in managing deeper biofilm in periodontal pockets,[14,21,24,26,49] the Er:YAG laser[14,18,31–36,38–42,45–48,50–53] has shown promise in disrupting surface biofilm and enhancing aesthetic or surface-level treatments.[57–61] Overall, integrating advanced visualization techniques, clinically relevant substrates and

combined laser systems will provide a more comprehensive understanding of biofilm responses to laser treatment and enhance the translational value of these findings for dental and medical applications.

Limitations

Despite the promising findings of this study, it is imperative to acknowledge its limitations. First, the study was conducted in vitro, which may not fully replicate the complex conditions present in vivo, where additional factors such as host immune responses, saliva and tissue interactions could influence the effectiveness of Er:YAG laser treatments on biofilm. The crystal violet staining method, a widely utilized technique for biofilm biomass quantification due to its simplicity and cost-effectiveness, is not without its limitations, which may affect its reliability. A significant drawback is its inability to differentiate between live and dead cells, as it quantifies total biomass without providing insights into cell viability or metabolic activity. This limitation may lead to an incomplete assessment of the functional state of biofilms. A study by Fernandes et al. emphasizes the importance of multiparametric approaches for more comprehensive evaluations.[62] Complementary methods such as live/dead staining, metabolic assays, or confocal microscopy could enhance reliability and address potential biases, including material–biofilm interactions. Future studies should consider these approaches to strengthen findings. Additionally, the study's focus on only 3 species, *C. albicans*, *C. glabrata* and *S. mutans*, limits the generalizability of the results to other pathogens commonly involved in oral infections. Furthermore, although the study demonstrated significant reductions in microbial populations, the long-term efficacy of the laser treatment was not assessed beyond the 24-hour post-irradiation time point. In clinical settings, the potential for biofilm regrowth necessitates the consideration of repeated treatments or combination therapies. The absence of a comprehensive analysis on the potential tissue damage or adverse effects of laser application also represents a limitation, as these factors are crucial in clinical applications. These limitations indicate that while Er:YAG lasers demonstrate potential for biofilm disruption, further research, particularly clinical studies, is needed to fully assess their effectiveness in diverse oral environments.

Conclusions

The present study aimed to evaluate the effects of the Er:YAG laser on biofilms composed of *C. albicans*, *C. glabrata* and *S. mutans* strains, with a particular focus on its antifungal and antibacterial properties. However, the findings are limited by the in vitro nature of the study, which does not fully replicate the physiological conditions of the oral environment. Consequently, the outcomes may not directly translate to clinical dentistry. The results suggest that the efficacy of laser treatment is influenced by several factors, including the fluence settings (0.4 J/cm^2 and 1.6 J/cm^2), microbial species and the time elapsed post-irradiation. Generally, the antifungal effects were evident in the reduction of fungal strains (*C. albicans* and *C. glabrata*) immediately after irradiation, with higher fluence settings showing greater efficacy. In contrast, the antibiofilm effect was characterized by sustained microbial reduction over a 24-hour period, particularly at lower fluence settings, with notable effectiveness against *C. glabrata* and *S. mutans* in single-species planktonic cultures. Future research should focus on in vivo studies to assess the laser's effectiveness under real-world conditions, including the complex interplay of oral microbiota, tissue responses and environmental factors.

Ethics approval and consent to participate

The study was approved by the Institutional Research Ethics Committee of Wroclaw Medical University, Poland (approval No. KB-429/2024).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

ChatGPT 4.0 (OpenAI) was used to verify grammar and make minor adjustments for clarity and accuracy.

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References

1. Kapila YL. Oral health's inextricable connection to systemic health: Special populations bring to bear multimodal relationships and factors connecting periodontal disease to systemic diseases and conditions. *Periodontol 2000*. 2021;87(1):11–16. doi:10.1111/prd.12398
2. Graves DT, Corrêa JD, Silva TA. The oral microbiota is modified by systemic diseases. *J Dent Res*. 2019;98(2):148–156. doi:10.1177/0022034518805739
3. Hu Q, Wang S, Zhang W, Qu J, Liu GH. Unraveling brain aging through the lens of oral microbiota. *Neural Regen Res*. 2025;20(7):1930–1943. doi:10.4103/NRR.NRR-D-23-01761

4. Slowik J, Wnuk MA, Grzech K, et al. Periodontitis affects neurological deficit in acute stroke. *J Neurol Sci*. 2010;297(1–2):82–84. doi:10.1016/j.jns.2010.07.012
5. Nomura R, Matayoshi S, Otsugu M, Kitamura T, Teramoto N, Nakano K. Contribution of severe dental caries induced by *Streptococcus mutans* to the pathogenicity of infective endocarditis. *Infect Immun*. 2020;88(7):e00897–e00919. doi:10.1128/IAI.00897-19
6. Fumes AC, da Silva Telles PD, Corona SAM, Borsatto MC. Effect of aPDT on *Streptococcus mutans* and *Candida albicans* present in the dental biofilm: Systematic review. *Photodiagnosis Photodyn Ther*. 2018;21:363–366. doi:10.1016/j.pdpdt.2018.01.013
7. Girois SB, Chapuis F, Decullier E, Revol BGP. Adverse effects of antifungal therapies in invasive fungal infections: Review and meta-analysis. *Eur J Clin Microbiol Infect Dis*. 2005;24(2):119–130. doi:10.1007/s10096-005-1281-2
8. Contaldo M, Di Stasio D, Romano A, et al. Oral candidiasis and novel therapeutic strategies: Antifungals, phytotherapy, probiotics, and photodynamic therapy. *Curr Drug Deliv*. 2023;20(5):441–456. doi:10.2174/1567201819666220418104042
9. Lewis MAO, Williams DW. Diagnosis and management of oral candidosis. *Br Dent J*. 2017;223(9):675–681. doi:10.1038/sj.bdj.2017.886
10. Millsop JW, Fazel N. Oral candidiasis. *Clin Dermatol*. 2016;34(4):487–494. doi:10.1016/j.clindermatol.2016.02.022
11. Shenoy A, Gottlieb A. Probiotics for oral and vulvovaginal candidiasis: A review. *Dermatol Ther*. 2019;32(4):e12970. doi:10.1111/dth.12970
12. Russo R, Superti F, Karadja E, De Seta F. Randomised clinical trial in women with recurrent vulvovaginal candidiasis: Efficacy of probiotics and lactoferrin as maintenance treatment. *Mycoses*. 2019;62(4):328–335. doi:10.1111/myc.12883
13. Matys J, Grzech-Leśniak K, Flieger R, Dominiak M. Assessment of an impact of a diode laser mode with wavelength of 980 nm on a temperature rise measured by means of k-02 thermocouple: Preliminary results. *Dent Med Probl*. 2016;53(3):345–351. doi:10.17219/dmp/62575
14. Grzech-Leśniak K. Making use of lasers in periodontal treatment: A new gold standard? *Photomed Laser Surg*. 2017;35(10):513–514. doi:10.1089/pho.2017.4323
15. Wiench R, Skaba D, Matys J, Grzech-Leśniak K. Efficacy of toluidine blue-mediated antimicrobial photodynamic therapy on *Candida* spp. A systematic review. *Antibiotics (Basel)*. 2021;10(4):349. doi:10.3390/antibiotics10040349
16. Wiench R, Nowicka J, Pajączkowska M, et al. Influence of incubation time on ortho-toluidine blue mediated antimicrobial photodynamic therapy directed against selected *Candida* strains – an in vitro study. *Int J Mol Sci*. 2021;22(20):10971. doi:10.3390/ijms222010971
17. Nammour S, El Mobadder M, Namour M, et al. Aesthetic treatment outcomes of capillary hemangioma, venous lake, and venous malformation of the lip using different surgical procedures and laser wavelengths (Nd:YAG, Er,Cr:YSGG, CO_2, and diode 980 nm). *Int J Environ Res Public Health*. 2020;17(22):8665. doi:10.3390/ijerph17228665
18. Elkharashi A, Grzech-Leśniak K, Deeb JG, Abdulmajeed AA, Bencharit S. Exploring the use of pulsed erbium lasers to retrieve a zirconia crown from a zirconia implant abutment. *PLoS One*. 2020;15(6):e0233536. doi:10.1371/journal.pone.0233536
19. El Mobadder M, Nammour S, Namour M, Namour A, Grzech-Leśniak K. Disinfection potential of 980 nm diode laser and hydrogen peroxide (3%) in "critical probing depths" periodontal pockets: Retrospective study. *Life (Basel)*. 2022;12(3):370. doi:10.3390/life12030370
20. Arnabat-Dominguez J, Del Vecchio A, Todea C, et al. Laser dentistry in daily practice during the COVID-19 pandemic: Benefits, risks and recommendations for safe treatments. *Adv Clin Exp Med*. 2021;30(2):119–125. doi:10.17219/acem/130598
21. Grzech-Leśniak K, Belvin BR, Lewis JP, Golob Deeb J. Treatment with Nd:YAG laser irradiation combined with sodium hypochlorite or hydrogen peroxide irrigation on periodontal pathogens: An in vitro study. *Photobiomodul Photomed Laser Surg*. 2021;39(1):46–52. doi:10.1089/photob.2019.4775
22. Grzech-Leśniak K, Nowicka J, Pajączkowska M, et al. Effects of Nd:YAG laser irradiation on the growth of *Candida albicans* and *Streptococcus mutans*: In vitro study. *Lasers Med Sci*. 2019;34(1):129–137. doi:10.1007/s10103-018-2622-6
23. Grzech-Leśniak Z, Szwach J, Lelonkiewicz M, et al. Effect of Nd:YAG laser irradiation on the growth of oral biofilm. *Microorganisms*. 2024;12(11):2231. doi:10.3390/microorganisms12112231
24. El Mobadder M, Nammour S, Grzech-Leśniak Z, Grzech-Leśniak K. Efficacy of the adjunct use of povidone-iodine or sodium hypochlorite with non-surgical management of periodontitis: A systematic review and meta-analysis. *J Clin Med*. 2022;11(21):6593. doi:10.3390/jcm11216593
25. Sterczała B, Grzech-Leśniak K, Michel O, Trzeciakowski W, Dominiak M, Jurczyszyn K. Assessment of human gingival fibroblast proliferation after laser stimulation in vitro using different laser types and wavelengths (1064, 980, 635, 450, and 405 nm) – preliminary report. *J Pers Med*. 2021;11(2):98. doi:10.3390/jpm11020098
26. Golob Deeb J, Reddy N, Kitten T, Carrico CK, Grzech-Leśniak K. Viability of bacteria associated with root caries after Nd:YAG laser application in combination with various antimicrobial agents: An in vitro study. *Dent Med Probl*. 2023;60(4):649–655. doi:10.17219/dmp/171690
27. Pion LA, Machado De Matos LL, Gimenez T, Palma-Dibb RG, Faraoni JJ. Treatment outcome for dentin hypersensitivity with laser therapy: Systematic review and meta-analysis. *Dent Med Probl*. 2023;60(1):153–166. doi:10.17219/dmp/151482
28. Torres-Rosas R, Torres-Gómez N, Camero-Leal JA, Jurado C, López-Ravelo H, Argueta-Figueroa L. Force decay and elongation of orthodontic elastomeric chains exposed to different beverages common in the diet: An in vitro study. *Dent Med Probl*. 2023;60(3):413–420. doi:10.17219/dmp/148052
29. Mosaddad SA, Mahootchi P, Rastegar Z, et al. Photodynamic therapy in oral cancer: A narrative review. *Photobiomodul Photomed Laser Surg*. 2023;41(6):248–264. doi:10.1089/photob.2023.0030
30. Uzeda MJ, Silva AM, Costa LN, Brito FS, Fernandes GV, Resende RF. Evaluating the effectiveness of low-level laser therapy in patients undergoing lower third molar extraction: A double-blinded randomized controlled trial. *Med Oral Patol Oral Cir Bucal*. 2025;30(1):e129–e134. doi:10.4317/medoral.26894
31. Golob Deeb J, Grzech-Lesniak K, Bencharit S. Evaluation of the effectiveness and practicality of erbium lasers for ceramic restoration removal: A retrospective clinical analysis. *PLoS One*. 2023;18(12):e0295957. doi:10.1371/journal.pone.0295957
32. Golob Deeb J, Grzech-Leśniak K, Brody ER, Matys J, Bencharit S. Erbium laser-assisted ceramic debonding: A scoping review. *J Prosthodont*. 2022;31(9):e100–e124. doi:10.1111/jopr.13613
33. Grzech-Leśniak K, Matys J. The effect of Er:YAG lasers on the reduction of aerosol formation for dental workers. *Materials (Basel)*. 2021;14(11):2857. doi:10.3390/ma14112857
34. Golob Deeb J, Skrjanc L, Kanduti D, Carrico C, Saturno AM, Grzech-Leśniak K. Evaluation of Er:YAG and Er,Cr:YSGG laser irradiation for the debonding of prefabricated zirconia crowns. *Adv Clin Exp Med*. 2021;30(1):7–15. doi:10.17219/acem/127686
35. Golob Deeb J, Grzech-Leśniak K, Weaver C, Matys J, Bencharit S. Retrieval of glass fiber post using Er:YAG laser and conventional endodontic ultrasonic method: An in vitro study. *J Prosthodont*. 2019;28(9):1024–1028. doi:10.1111/jopr.13114
36. Grzech-Leśniak K, Bencharit S, Dalal N, Mroczka K, Golob Deeb J. In vitro examination of the use of Er:YAG laser to retrieve lithium disilicate crowns from titanium implant abutments. *J Prosthodont*. 2019;28(6):672–676. doi:10.1111/jopr.13077
37. Rubio F, Arnabat-Domínguez J, Sans-Serramitjana E, Saa C, Grzech-Leśniak K, Betancourt P. Antimicrobial photodynamic therapy combined with photobiomodulation therapy in teeth with asymptomatic apical periodontitis: A case series. *Appl Sci*. 2024;14(20):9341. doi:10.3390/app14209341
38. Nagahashi T, Yahata Y, Handa K, et al. Er:YAG laser-induced cavitation can activate irrigation for the removal of intraradicular biofilm. *Sci Rep*. 2022;12(1):4897. doi:10.1038/s41598-022-08963-x
39. Nammour S, El Mobadder M, Namour M, et al. Success rate of direct pulp capping with conventional procedures using Ca $(OH)_2$ and bioactive tricalcium silicate paste vs. laser-assisted procedures (diode 980 nm, CO_2, and Er: YAG). *Photonics*. 2023;10(7):834. doi:10.3390/photonics10070834
40. AlMoharib HS, Steffensen B, Zoukhri D, Finkelman M, Gyurko R. Efficacy of an Er:YAG laser in the decontamination of dental implant surfaces: An in vitro study. *J Periodontol*. 2021;92(11):1613–1621. doi:10.1002/JPER.20-0765

41. Vezzani MS, Pietro R, Silva-Sousa YT, Brugnera-Junior A, Sousa-Neto MD. Disinfection of root canals using Er:YAG laser at different frequencies. *Photomed Laser Surg*. 2006;24(4):499–502. doi:10.1089/pho.2006.24.499
42. Świder K, Dominiak M, Grzech-Leśniak K, Matys J. Effect of different laser wavelengths on periodontopathogens in peri-implantitis: A review of in vivo studies. *Microorganisms*. 2019;7(7):189. doi:10.3390/microorganisms7070189
43. Matys J, Świder K, Grzech-Leśniak K, Dominiak M, Romeo U. Photobiomodulation by a 635nm diode laser on peri-implant bone: Primary and secondary stability and bone density analysis – a randomized clinical trial. *Biomed Res Int*. 2019;2019:2785302. doi:10.1155/2019/2785302
44. Hou X, Yuan K, Huang Z, Ma R. Effects of bleaching associated with Er:YAG and Nd:YAG laser on enamel structure and bacterial biofilm formation. *Scanning*. 2021;2021:6400605. doi:10.1155/2021/6400605
45. Grzech-Leśniak K, Matys J, Jurczyszyn K, et al. Histological and thermometric examination of soft tissue de-epithelialization using digitally controlled Er:YAG laser handpiece: An ex vivo study. *Photomed Laser Surg*. 2018;36(6):313–319. doi:10.1089/pho.2017.4413
46. Grzech-Leśniak K, Matys J, Dominiak M. Comparison of the clinical and microbiological effects of antibiotic therapy in periodontal pockets following laser treatment: An in vivo study. *Adv Clin Exp Med*. 2018;27(9):1263–1270. doi:10.17219/acem/70413
47. Golob Deeb J, Smith J, Ross Belvin B, Lewis J, Grzech-Leśniak K. Er:YAG laser irradiation reduces microbial viability when used in combination with irrigation with sodium hypochlorite, chlorhexidine, and hydrogen peroxide. *Microorganisms*. 2019;7(12):612. doi:10.3390/microorganisms7120612
48. Sennhenn-Kirchner S, Schwarz P, Schliephake H, Konietschke F, Brunner E, Borg-von Zepelin M. Decontamination efficacy of erbium:yttrium-aluminium-garnet and diode laser light on oral *Candida albicans* isolates of a 5-day in vitro biofilm model. *Lasers Med Sci*. 2009;24(3):313–320. doi:10.1007/s10103-008-0561-3
49. El Mobadder M, Nammour S, Matys J, Grzech-Leśniak K. Sodium hypochlorite and diode laser in non-surgical treatment of periodontitis: Clinical and bacteriological study with real time polymerase chain reaction (PCR). *Life (Basel)*. 2022;12(10):1637. doi:10.3390/life12101637
50. Reddy N, Golob Deeb J, Kitten T, Carrico CK, Grzech-Leśniak K. The in vitro effect of laser irradiation (Er:YAG and CO_2) and chemical reagents (hydrogen peroxide, sodium hypochlorite, chlorhexidine, or sodium fluoride) alone or in combination on reducing root caries bacteria. *Int J Mol Sci*. 2022;23(24):15732. doi:10.3390/ijms232415732
51. Henninger E, Berto LA, Eick S, Lussi A, Neuhaus KW. In vitro effect of Er:YAG laser on different single and mixed microorganisms being associated with endodontic infections. *Photobiomodul Photomed Laser Surg*. 2019;37(6):369–375. doi:10.1089/photob.2018.4557
52. Bordea IR, Hanna R, Chiniforush N, et al. Evaluation of the outcome of various laser therapy applications in root canal disinfection: A systematic review. *Photodiagnosis Photodyn Ther*. 2020;29:101611. doi:10.1016/j.pdpdt.2019.101611
53. Polak D, Shani-Kdoshim S, Alias M, Shapira L, Stabholz A. The in vitro efficacy of biofilm removal from titanium surfaces using Er:YAG laser: Comparison of treatment protocols and ablation parameters. *J Periodontol*. 2022;93(1):100–109. doi:10.1002/JPER.19-0574
54. Liddell LT, Rosen T. Laser therapy for onychomycosis: Fact or fiction? *J Fungi (Basel)*. 2015;1(1):44–54. doi:10.3390/jof1010044
55. Salehi B, Kregiel D, Mahady G, Sharifi-Rad J, Martins N, Rodrigues CF. Management of *Streptococcus mutans-Candida* spp. oral biofilms' infections: Paving the way for effective clinical interventions. *J Clin Med*. 2020;9(2):517. doi:10.3390/jcm9020517
56. Misischia WP, Xenoudi P, Yukna RA, Schurr MJ. Bacterial reduction effect of four different dental lasers on titanium surfaces in vitro. *Lasers Med Sci*. 2021;36(8):1759–1767. doi:10.1007/s10103-021-03349-3
57. Wang X, Yuan C, Wo Y, et al. Will repeated ablative Er:YAG laser treatment sessions cause facial skin sensitivity? Results of a 12-month, prospective, randomized split-face study. *Rejuvenation Res*. 2020;23(2):122–129. doi:10.1089/rej.2018.2168
58. Medved F, Wurm A, Held M. Facial microcirculatory and biomechanical skin properties after single high energy (Er):YAG laser application. *Lasers Surg Med*. 2017;49(10):891–898. doi:10.1002/lsm.22710
59. Holcomb JD. Versatility of erbium YAG laser: From fractional skin rejuvenation to full-field skin resurfacing. *Facial Plast Surg Clin North Am*. 2011;19(2):261–273. doi:10.1016/j.fsc.2011.04.005
60. Frelich H, Marków M, Tażbirek M, Frelich-Truchel H, Misiołek M, Ścierski W. Erbium:yttrium aluminum garnet (Er:YAG) laser: A minimally invasive treatment method in selected patients with impaired breathing during sleep – the assessment of treatment effectiveness after 4 years. *Photobiomodul Photomed Laser Surg*. 2023;41(8):415–421. doi:10.1089/photob.2022.0144
61. Storchi IF, Parker S, Bovis F, Benedicenti S, Amaroli A. Outpatient erbium:YAG (2940 nm) laser treatment for snoring: A prospective study on 40 patients. *Lasers Med Sci*. 2018;33(2):399–406. doi:10.1007/s10103-018-2436-6
62. Fernandes GVO, Alves GG, Linhares ABR, Prado da Silva MH, Granjeiro JM. Evaluation of cytocompatibility of bioglass-niobium granules with human primary osteoblasts: A multiparametric approach. *Key Eng Mater*. 2011;493–494:37–42. doi:10.4028/www.scientific.net/kem.493-494.37

Original paper

Influence of thermal aging on surface roughness of conventional and 3D-printed materials used in intraoral appliance manufacturing

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Abstract

Background. Intraoral appliances are widely used in dentistry. Their surface roughness may influence patient comfort, biofilm formation and durability.

Objectives. The aim of the study was to evaluate and compare the effect of thermocycling on the surface roughness (Ra) of different materials used for the fabrication of intraoral appliances.

Material and methods. Seventy-two standardized specimens (40 mm × 10 mm × 2 mm) were fabricated from 3 materials: a self-curing poly(methyl methacrylate) (PMMA) resin (PMMA group); a light-cured urethane dimethacrylate (UDMA)-based resin (UDMA group); and a stereolithography (SLA) 3D-printed resin (SLA group). Surface roughness was measured before and after thermocycling (5,000 and 10,000 cycles between 5°C and 55°C) using a contact profilometer. Values were reported as mean (*M*) ±standard deviation (*SD*). The data was analyzed using repeated-measures or ordinary one-way analysis of variance (ANOVA), followed by Bonferroni-corrected post hoc tests ($a = 0.0167$).

Results. The UDMA group exhibited the lowest mean initial Ra values (0.078 ±0.020 µm). Thermocycling induced changes in surface roughness. In the PMMA group, a significant increase in mean Ra was observed after 5,000 cycles (0.103 ±0.028 µm before vs. 0.167 ±0.059 µm after; $p = 0.0001$) and after 10,000 cycles (0.107 ±0.024 µm before vs. 0.205 ±0.060 µm after; $p < 0.0001$). The increase in mean Ra following thermocycling was significantly greater in the PMMA group compared to the other groups (mean ΔRa after 5,000 cycles: 0.064 ±0.035 µm; after 10,000 cycles: 0.098 ±0.046 µm; all $p < 0.0001$).

Conclusions. Materials used for the fabrication of intraoral appliances exhibit material-specific responses to thermal aging. The light-cured UDMA-based resin demonstrated superior surface integrity after aging, whereas conventional PMMA and the 3D-printed resin were more susceptible to surface alterations.

Keywords: PMMA, surface roughness, UDMA, intraoral appliance, 3D-printed resin

Highlights

- The light-cured urethane dimethacrylate (UDMA)-based resin demonstrated superior surface integrity after thermal aging, while conventional poly(methyl methacrylate) (PMMA) and the 3D-printed resin showed greater susceptibility to surface degradation.
- Despite these differences, all materials maintained surface roughness values below the critical threshold for bacterial adhesion (0.2 µm).
- PMMA and the 3D-printed resin exhibited a significant increase in surface roughness during use, indicating a greater need for repolishing compared with the UDMA-based resin.
- Clinical selection of intraoral appliance materials should consider additional properties, including mechanical strength and wear resistance.

Introduction

Intraoral appliances, such as occlusal splints and mandibular advancement devices, are widely used in dentistry to manage temporomandibular disorders (TMD), protect dental hard tissues, reduce bruxism symptoms, and treat obstructive sleep apnea.[1–3] These intraoral devices create a physical barrier between the dental arches, provide suitable occlusal contacts, redistribute occlusal forces, reduce muscle activity, protect temporomandibular joint structures, and decrease the frequency of apnea episodes.[1–4] Recent epidemiological studies indicate that approx. 8–12% of the adult population requires some form of occlusal therapy, highlighting the clinical relevance of these appliances.[5]

The efficacy of occlusal splint therapy and patient compliance depend on proper design and adjustment, as well as on the physical and mechanical properties of the materials used for fabrication.[6] Traditionally, poly(methyl methacrylate) (PMMA) has been the material of choice due to its favorable mechanical properties, ease of manipulation and cost-effectiveness.[7] However, advancements in dental material science have introduced alternative materials, including light-cured urethane dimethacrylate (UDMA) resins and 3D-printable resins, which are increasingly adopted in clinical practice.[8–10]

Surface roughness (Ra) is a critical physical property of dental materials that has an influence on biofilm formation, staining potential, wear resistance, and patient comfort.[11] Quirynen et al. demonstrated that surfaces with an Ra exceeding 0.2 µm significantly increase bacterial adhesion and biofilm formation.[12] Furthermore, Ionescu et al. reported that increased Ra of intraoral appliances may cause tongue irritation, altered taste perception and reduced patient compliance.[13] From a longevity perspective, rougher surfaces are more susceptible to wear and chemical degradation, potentially compromising the mechanical integrity and therapeutic efficacy of occlusal splints during clinical service.[14]

The oral environment exposes dental materials to numerous physical and chemical challenges, including temperature fluctuations, pH variations, masticatory forces, and salivary enzymes.[15] Artificial aging protocols, such as thermocycling, are employed to predict the long-term performance of dental materials in laboratory settings.[16] Thermocycling simulates thermal changes in the oral cavity through repeated exposure to temperature extremes, typically ranging from 5°C to 55°C.[17] This process induces structural stress within materials due to differences in thermal expansion coefficients among their components, which may affect surface characteristics and mechanical properties.[18] Although previous studies have extensively investigated the effects of artificial aging on the mechanical properties of occlusal splint materials,[19] evidence regarding its influence on Ra remains limited and often contradictory.[20]

The emergence of digital technologies in dentistry, particularly computer-aided design and computer-aided manufacturing (CAD/CAM) and 3D printing, has been hoped to revolutionize the fabrication process of occlusal splints.[21] Additive manufacturing techniques such as stereolithography (SLA) offer advantages including high precision, reproducibility and time efficiency.[22] However, the long-term performance of 3D-printed materials in the oral environment, especially regarding surface properties after aging, has not been sufficiently documented.[23] Revilla-León et al. highlighted the need for comprehensive investigations evaluating the aging behavior of these novel materials compared with conventional alternatives.[24]

Furthermore, the effect of thermocycling on Ra may vary significantly among materials used for the fabrication of intraoral appliances. Factors such as polymer composition, cross-linking density, filler content, and manufacturing techniques may influence susceptibility to thermal degradation and subsequent surface alterations.[25] Identifying material-specific responses to artificial aging would provide valuable guidance for clinicians in selecting the most appropriate material for individual clinical situations.[26]

Despite the clinical importance of Ra for the performance and longevity of intraoral appliances, standardized comparative studies evaluating the response of different

contemporary materials to artificial aging protocols remain scarce. This knowledge gap is particularly evident for newer 3D-printable resins, which are increasingly incorporated into routine dental practice.[23,26,27]

Therefore, the aim of this study was to evaluate and compare the influence of artificial aging on the Ra of 3 different materials commonly used for intraoral appliance fabrication: a self-curing PMMA resin; a light-cured UDMA-based resin; and an SLA 3D-printed resin. The null hypotheses were that thermocycling would not significantly affect Ra, and that no differences would be observed between the materials in their response to thermal aging. The findings of this investigation may provide evidence-based guidance for material selection in the fabrication of intraoral appliances, potentially improving clinical performance and patient satisfaction.

Material and methods

Materials

This in vitro study evaluated 3 materials commonly used for the fabrication of intraoral appliances. These materials were selected based on their widespread clinical application, distinct chemical compositions and different manufacturing methodologies, as reported in contemporary dental literature.[28] Additionally, the selection reflected the authors' extensive clinical experience with these materials.

The first material tested was a classic self-curing PMMA resin (Estetic Ort; Wiedent, Łódź, Poland) (PMMA group). This material represents the conventional approach to intraoral appliance fabrication and consists of separate powder and liquid components. It served as the control and reference material for intermaterial comparisons.

The second material evaluated was a light-cured UDMA-based resin (DURASPLINT® LC; Scheu Dental, Iserlohn, Germany) (UDMA group). This product is supplied as preformed bars and consists primarily of a UDMA matrix with minor additions of acrylic resin, photoinitiators and cross-linking agents.

The third material investigated was a biocompatible photopolymer specifically designed for SLA 3D printing (Dental LT Clear V1; Vertex Dental, Soesterberg, Netherlands) (SLA group). This product is classified as a Class IIa biocompatible resin composed mainly of methacrylate oligomers, methacrylate monomers and photoinitiators. Dental LT Clear is approved for the fabrication of orthodontic and dental devices, including occlusal splints.

Specimen preparation

A total of 72 standardized rectangular specimens were fabricated (n = 24 per group). The specimen dimensions (40 mm × 10 mm × 2 mm) were selected following the recommendations reported in similar studies.[29,30]

The PMMA specimens were prepared using a standardized compression molding technique with metal molds to ensure dimensional accuracy.[29–31] The material was mixed according to the manufacturer's instructions, with a standardized powder-to-liquid ratio of 3:1 by weight. The mixture was packed into the molds at the dough-like stage, compressed between glass plates under 2.0 bar pressure, and allowed to polymerize for 20 min at room temperature (23 ±1°C), followed by 30 min in a pressure vessel (Polyclav®; Dentaurum, Ispringen, Germany) at 55°C and 2.5 bar pressure.[30]

To ensure dimensional accuracy, the UDMA-based specimens were prepared using calibrated silicone molds and a compression technique. Polymerization was performed in a laboratory light-curing unit (LC-6 Light Oven; Scheu Dental) for 2 cycles of 10 min each. The specimens were inverted halfway through the process to ensure complete polymerization of both surfaces.[32]

The SLA specimens were designed using Meshmixer v. 3.5.474 (https://meshmixer.org; Autodesk, San Francisco, USA). Subsequently, the PreForm® software v. 3.28.1 (https://formlabs.com/software/preform; Formlabs Inc., Somerville, USA) was used to generate supports and define printing parameters. The specimens were printed using a 3D printer (Form 2; Formlabs Inc.) at a resolution of 100 µm, with layers oriented at 90° to the building platform. After printing, the specimens were washed twice for 10 min in 99% isopropanol (STANLAB, Lublin, Poland) and air-dried at room temperature for 30 min. Subsequently, they were post-cured in a Form Cure (Formlabs Inc.) at 80°C for 20 min. Finally, the supports were removed by cutting and grinding.[32]

Following fabrication, all specimens were inspected for defects such as porosities, inclusions or dimensional irregularities. Specimens exhibiting any visible defects were excluded and replaced. To remove processing artifacts, specimen edges were polished with 1,000-grit silicon carbide paper (P.S. Trading, Ołtarzew, Poland). Water sandpaper (grit P500, P1000, P1200; P.S. Trading) and 0.6-mm pumice stone powder (Everall7, Warsaw, Poland) were used to finish the specimens. The upper surface of each specimen was polished for 1 min with a polishing paste for resin and metals (Everall7) using POLIRET MINI (REITEL Feinwerktechnik GmbH, Bad Essen, Germany). The procedure was conducted in accordance with the manufacturer's instructions and available scientific literature regarding chosen materials.[32,33] All polishing procedures were performed by the same experienced operator to minimize variability. Finally, each specimen was rinsed under water and underwent ultrasonic cleaning (Elmasonic S30H; Elma Schmidbauer GmbH, Singen, Germany) in distilled water with a mild detergent (CLEAN and CLEVER; IGEFA Handelsgesellschaft mbH & Co. KG, Ahrensfelde, Germany) at 30°C for 10 min.

Conditioning

All specimens were stored in artificial saliva (UKD saliva solution; University Pharmacy, Dresden, Germany) for 72 h at 37°C to simulate the intraoral environment and allow water sorption equilibrium.[29,32–35]

Subsequently, the specimens were transferred to distilled water for an additional 48 h at 37°C.[36] All specimens underwent ultrasonic cleaning under the conditions described above to remove surface contaminants.[37,38]

Thermal aging

Specimens from each group were randomly divided into 2 equal subgroups (n = 12 per subgroup) according to thermocycling exposure (5,000 or 10,000 cycles). Thermal aging was performed using a thermocycler (THE-1200; SD Mechatronik GmbH, Feldkirchen-Westerham, Germany). The specimens were alternately immersed in distilled water at 5°C and 55°C. Each cycle lasted 77 s, with 27 s in each liquid container and 9-s drip time.[29,39] A total of 5,000 and 10,000 cycles were performed to simulate 6 and 12 months of clinical use, respectively.[29,40] However, it should be noted that the cycle-to-time correlation was an estimate for comparative purposes only.

Surface roughness measurements

Surface roughness measurements were performed after specimen preparation (initial), after conditioning (baseline) and after thermal aging (5,000 or 10,000 cycles). Surface roughness was measured using a contact profilometer (Hommel-Etamic W20; JENOPTIK Industrial Metrology Germany GmbH, Villingen-Schwenningen, Germany) with the stylus method in accordance with the VDE standard VDI/VDE 2602 Part 2, 2018-04: Surface Measurement.[41] For each specimen, 6 measurements were taken: 3 longitudinal and 3 transverse, positioned perpendicular to each other. The angle of measurement for the diagonal printing path was 45°. The measurements were conducted with a stylus tip radius of 2 µm and a tip angle of 90°. The feed mechanism moved horizontally along the polished surface at a constant speed of 0.5 mm/s and a measuring force of approx. 0.8 mN. The measuring length was 4.8 mm, with a cut-off wavelength of 0.8 mm. Subsequently, the arithmetic mean Ra value was calculated from the 6 measurements for each specimen at each time point (initial, baseline, and 5,000 or 10,000 cycles) within the thermal aging process.[29,30] The change in Ra (ΔRa) was calculated as the difference between baseline Ra and Ra after thermal aging (5,000 or 10,000 cycles).

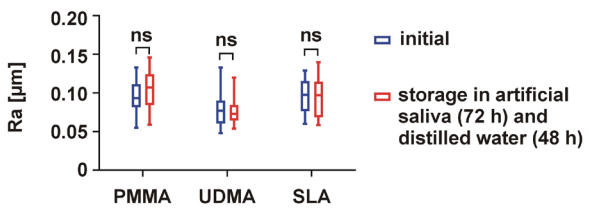

Fig. 1. Box plot presenting the surface roughness (Ra) of the evaluated materials at the initial time point (after specimen preparation) and after storage in artificial saliva (72 h) followed by distilled water (48 h)

PMMA – self-curing poly(methyl methacrylate) resin; UDMA – light-cured urethane dimethacrylate-based resin; SLA – stereolitography 3D-printed resin; ns – not significant.

Statistical analysis

The results were analyzed using the GraphPad Prism 9.1.2. software (GraphPad Software, Boston, USA). Descriptive statistics were presented as box plots showing the five-number summary of data (minimum, first quartile, median, third quartile, and maximum) (n = 12 for each group) and as mean (M) ±standard deviation (SD) in the text. Normality of data distribution was assessed using the Shapiro–Wilk test, which confirmed normal distribution. For within-material comparison, differences in Ra across time points were analyzed using repeated measures one-way analysis of variance (ANOVA) followed by Bonferroni-corrected post hoc tests (α = 0.0167). For between-material comparison, ΔRa values after 5,000 and 10,000 thermocycles were analyzed separately using one-way ANOVA followed by Bonferroni-corrected post hoc tests (α = 0.0167).

Results

The descriptive data for the initial Ra values and Ra after conditioning (baseline) is shown in Fig. 1. The UDMA group exhibited the lowest mean initial Ra values (0.078 ±0.020 µm), whereas the SLA group showed the highest mean Ra values (0.096 ±0.020 µm). The coefficient of variation for mean Ra within each material group ranged from 13.314% (UDMA) to 28.992% (SLA). After storing the specimens in artificial saliva and distilled water, no significant changes in Ra were observed for any of the tested materials (all $p > 0.05$) (Fig. 1). The coefficient of variation after conditioning ranged from 15.385% (UDMA) to 23.736% (PMMA). A comparison of ΔRa values after storage in artificial saliva and water revealed no statistically significant differences between the materials (Table 1).

The effects of thermocycling on Ra are presented in Fig. 2 and Table 2. An increase in mean Ra was observed for all materials after thermocycling; however, statistically significant changes were detected only in selected groups. After 5,000 thermocycles, significant increases in Ra were observed for PMMA (0.103 ±0.028 µm before and 0.167 ±0.059 µm after 5,000 cycles; p = 0.0001) and SLA (0.095 ±0.026 µm before and 0.110 ±0.021 µm after 5,000 cycles; p = 0.0006). No significant difference was

Table 1. Changes in surface roughness (ΔRa) of the evaluated materials after storage in artificial saliva (72 h) followed by distilled water (48 h), compared with the initial time point (after specimen preparation)

Variable	Groups compared (1 vs. 2)	Mean 1	Mean 2	Mean difference	95% *CI* of difference	*t*	*df*	Summary	*p*-value
ΔRa after conditioning	PMMA vs. UDMA	0.01067	−0.00025	0.01092	−0.00107–0.02290	2.235	69	ns	0.0860
	PMMA vs. SLA	0.01067	−0.00067	0.01133	−0.00065–0.02332	2.320	69	ns	0.0699
	UDMA vs. SLA	−0.00025	−0.00067	0.00042	−0.01157–0.01240	0.085	69	ns	>0.9999

Differences in mean Ra values between the materials were analyzed using repeated measures one-way ANOVA followed by Bonferroni-corrected post hoc tests ($\alpha = 0.0167$).
CI – confidence interval; *t* – test statistic; *df* – degrees of freedom; PMMA – self-curing poly(methyl methacrylate) resin; UDMA – light-cured urethane dimethacrylate-based resin; SLA – stereolitography 3D-printed resin; ns – not significant.

Table 2. Changes in surface roughness (ΔRa) of the evaluated materials after thermocycling

Variable	Groups compared (1 vs. 2)	Mean 1	Mean 2	Mean difference	95% *CI* of difference	*t*	*df*	*p*-value
ΔRa after 5,000 cycles	PMMA vs. UDMA	0.06433	0.00625	0.05808	0.03611–0.08006	6.667	33	<0.0001*
	PMMA vs. SLA	0.06433	0.01508	0.04925	0.02728–0.07122	5.653	33	<0.0001*
	UDMA vs. SLA	0.00625	0.01508	−0.00883	−0.03081–0.01314	1.014	33	0.9541
ΔRa after 10,000 cycles	PMMA vs. UDMA	0.09825	0.00167	0.09658	0.06624–0.12690	8.028	33	<0.0001*
	PMMA vs. SLA	0.09825	0.02442	0.07383	0.04349–0.10420	6.137	33	<0.0001*
	UDMA vs. SLA	0.00167	0.02442	−0.02275	−0.05310–0.00760	1.891	33	0.2023

Differences in mean Ra values between the materials were analyzed using repeated measures one-way ANOVA followed by Bonferroni-corrected post hoc tests ($\alpha = 0.0167$).
* statistically significant ($p < 0.0001$).

found for UDMA ($p > 0.05$). The coefficient of variation of the mean Ra ranged from 18.232% (SLA) to 34.735% (PMMA). A comparison of non-aged specimens with specimens subjected to 10,000 thermocycles demonstrated significant differences for PMMA (Ra = 0.107 ±0.024 µm before and Ra = 0.205 ±0.060 µm after 10,000 cycles; $p < 0.0001$) and SLA (Ra = 0.096 ±0.028 µm before and Ra = 0.121 ±0.022 µm after 10,000 cycles; $p = 0.002$), whereas no significant changes were detected for UDMA ($p > 0.05$) (Fig. 2). The coefficient of variation ranged from 17.465% (UDMA) to 29.452% (PMMA). Between-material comparison using one-way ANOVA demonstrated statistically significant differences in ΔRa values after thermocycling ($p < 0.0001$). Post hoc multiple comparisons revealed that the increase in the mean Ra after both 5,000 and 10,000 cycles was significantly higher in the PMMA group than in the UDMA and SLA groups (all $p < 0.0001$). No significant differences in Ra were observed between the UDMA and SLA groups after 5,000 cycles ($p = 0.954$) or 10,000 cycles ($p = 0.202$) (Table 2).

Discussion

The present study investigated the influence of artificial aging on Ra of 3 materials commonly used for the fabrication of intraoral appliances: a self-curing PMMA resin; a light-cured UDMA-based resin; and an SLA 3D-printed resin. The results demonstrated material-specific responses to thermal aging, leading to the rejection of both null hypotheses.

Initial measurements revealed significant differences in Ra among the tested materials, with the light-cured UDMA-based resin exhibiting the lowest Ra values. This finding is consistent with the results reported by Mahross et al., who demonstrated that light-cured UDMA resins typically present smoother surfaces than conventional PMMA, potentially due to differences in manufacturing processes and monomer composition.[42] The photopolymerization mechanism in UDMA-based materials may allow for more homogeneous polymerization with fewer internal defects, potentially contributing to improved surface characteristics.[43,44] Furthermore,

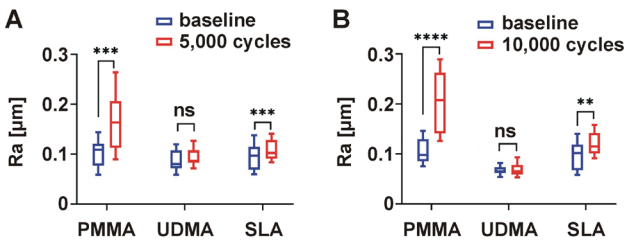

Fig. 2. Box plots illustrating the surface roughness (Ra) of the evaluated materials before and after thermocycling

A. Comparison between baseline values (after conditioning) and values obtained after 5,000 thermocycles; B. Comparison between baseline values (after conditioning) and values obtained after 10,000 thermocycles. ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

the preformed bar presentation of light-cured UDMA-based resin eliminates the need for mixing components, reducing the risk of incorporating air bubbles or contaminants that may compromise surface integrity. On the other hand, Weżgowiec et al., in a study comparing the biocompatibility of 3D-printed, thermoformed and heat-cured intraoral appliances, reported no statistically significant differences in Ra between polished self-curing PMMA, light-cured UDMA-based, and SLA 3D-printed resins.[33]

Storage in artificial saliva followed by distilled water did not significantly influence Ra of tested materials in the present study. However, previous research has demonstrated the hydrophilic nature of PMMA and its relatively high water sorption capacity.[45–47] Conventional PMMA undergoes hydrolytic degradation when exposed to aqueous environments, leading to monomer leaching and disruption of the polymer network.[48,49] Bettencourt et al. reported significant surface alterations in PMMA after storage in artificial saliva, attributing these changes to the combined effect of water sorption and the action of saliva on residual monomers.[50] With regard to the SLA 3D-printed resin, the findings reported in this study are consistent with other reports indicating that similar storage conditions did not significantly affect the Ra of Dental LT Clear.[51]

Thermocycling induced material-specific changes in Ra, with the self-curing PMMA resin exhibiting the most pronounced increase after both 5,000 and 10,000 cycles. This deterioration in surface quality can be attributed to several factors. First, PMMA has a relatively high coefficient of thermal expansion compared to other dental polymers, making it more susceptible to dimensional changes under temperature fluctuations.[52,53] Second, the powder–liquid mixing process in self-curing PMMA may result in heterogeneous polymerization, residual monomer content and porosities, creating weak points vulnerable to thermal stress.[54,55]

Similarly to PMMA, the SLA 3D-printed resin demonstrated significant increases in Ra after both 5,000 and 10,000 cycles. The layer-by-layer manufacturing process inherent to SLA technology may create microscopic interfaces between polymerized layers that become weak points during thermal cycling.[56] This finding is particularly relevant considering the growing popularity of 3D printing in dental applications. Time-dependent degradation in 3D-printed dental resins can be attributed to polymerization boundaries between layers, which become more evident over time.[57] It is worth emphasizing that printing parameters, such as orientation, have been shown to influence roughness and gloss of 3D-printed resins, suggesting that optimization of these parameters may enhance surface integrity.[58] Artificial aging has also been reported to affect other mechanical properties of Dental LT Clear, including compressive and tensile strength.[59] However, polishing procedures appear to mitigate aging-related damage.[59] Moreover, flexural strength and dimensional stability of other 3D-printed resins used in occlusal splint manufacturing have been reported to depend on printing and post-polymerization protocols.[60] Collectively, these findings underscore the need for further research to optimize protocols for 3D-printed resins.

In contrast, the light-cured UDMA-based resin demonstrated remarkable stability after thermal aging, with no significant changes in Ra even after 10,000 cycles. This enhanced resistance to thermal degradation can be explained by the high degree of conversion achieved through controlled light polymerization, resulting in a more densely cross-linked and stable polymer network.[43,44] Additionally, the absence of residual monomer, which typically acts as a plasticizer in self-curing resins, contributes to improved thermal stability of UDMA-based materials.[61]

From a clinical perspective, a Ra threshold of 0.2 μm has been established as critical for bacterial adhesion, with rougher surfaces promoting microbial colonization and biofilm formation.[62] In the present study, all materials maintained mean Ra values below this threshold even after simulating 1 year of use (10,000 thermocycles), suggesting acceptable clinical performance regarding microbial resistance. However, the significant increase in roughness observed for self-curing PMMA raises concerns about its long-term performance without additional polishing, particularly in patients with compromised oral hygiene or increased susceptibility to candidiasis.[63]

The different responses to artificial aging observed among the tested materials have important implications for clinical material selection. Particularly, the significant increase in Ra observed for PMMA and the 3D-printed resin during simulated long-term use suggests that these materials may require more frequent polishing compared with UDMA-based resins. In our previous study, polishing significantly reduced surface roughness parameters (both Ra and roughness depth (Rz)) in conventional PMMA and 3D-printed resins.[33] However, clinical decision-making should balance surface characteristics with other relevant material properties, such as mechanical strength and wear resistance.

Several limitations of the present study should be acknowledged. Firstly, although thermocycling is a widely accepted method for simulating thermal aging, it does not fully replicate the complex aging processes of dental materials in the oral environment, including masticatory forces, pH fluctuations and enzymatic activities. Moreover, the cycle-to-time correlation is an estimate for comparative purposes only, not a definitive predictor of clinical longevity. Secondly, Ra reflects only one aspect of material performance. Additional parameters, such as hardness, tensile strength and wear resistance, would allow for a more comprehensive evaluation of aging behavior.[59,60,64,65] Furthermore, the study evaluated only 3 commercially available materials, which may limit

the generalizability of the findings across broader material categories. Also, the specimen geometry used in this study (flat rectangles) differs from the complex three-dimensional structure of actual occlusal splints, potentially influencing aging patterns.

Despite these limitations and considering the limited available literature as well as the rapidly developing fields of TMD management and dental sleep medicine, where both traditional and digitally manufactured intraoral appliances are increasingly used, the present study provides a significant contribution to the field and a foundation for further research.

Future research should address these limitations by incorporating additional aging parameters. Studies evaluating the combined effect of thermal aging and mechanical stress would be particularly valuable, as occlusal splints are subjected to considerable forces during clinical use. In this context, it would also be interesting to investigate the mechanical surface wear of splint materials, taking into consideration chewing forces, and to determine the abrasion of microplastic particles. Comparative studies in a chewing simulator could provide results regarding microplastic particles that are currently the focus of attention due to their harmful effects on general health. Furthermore, exploring the relationship between surface changes and other clinically relevant outcomes, such as bacterial adhesion, staining susceptibility and patient comfort, would provide more comprehensive guidance for material selection. Despite the broad use and popularity of the materials selected for the purpose of this study, future studies should include a broader range of commercially available materials within each category to better assess in-group differences and improve the representativeness of results.

Long-term clinical trials comparing the performance of different splint materials would be the gold standard for validating laboratory findings and establishing evidence-based recommendations. Additionally, the development and evaluation of novel surface treatments or modifications aimed at improving the aging resistance of conventional PMMA and 3D-printed resins represent promising directions for future research.

Conclusions

The materials investigated for intraoral appliance fabrication exhibited significantly different responses to thermal aging. The light-cured UDMA-based resin demonstrated superior surface integrity, even after 10,000 thermal cycles. At the same time, the conventional self-curing PMMA and SLA 3D-printed resins showed greater susceptibility to thermal degradation, as reflected by significant increases in Ra. Despite these differences, all materials maintained Ra values below the clinically relevant threshold for bacterial adhesion (0.2 μm).

Ethics approval and consent to participate

Not applicable.

Data availability

The additional datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Orzeszek S, Waliszewska-Prosol M, Ettlin D, et al. Efficiency of occlusal splint therapy on orofacial muscle pain reduction: A systematic review. *BMC Oral Health*. 2023;23(1):180. doi:10.1186/s12903-023-02897-0
2. Martynowicz H, Wieczorek T, Macek P, et al. The effect of continuous positive airway pressure and mandibular advancement device on sleep bruxism intensity in obstructive sleep apnea patients. *Chron Respir Dis*. 2022;19:14799731211052301. doi:10.1177/14799731211052301
3. Kuzmanovic Pficer J, Dodic S, Lazic V, Trajkovic G, Milic N, Milicic B. Occlusal stabilization splint for patients with temporomandibular disorders: Meta-analysis of short and long term effects. *PLoS One*. 2017;12(2):e0171296. doi:10.1371/journal.pone.0171296
4. Fricton J, Look JO, Wright E, et al. Systematic review and meta-analysis of randomized controlled trials evaluating intraoral orthopedic appliances for temporomandibular disorders. *J Orofac Pain*. 2010;24(3):237–254. PMID:20664825.
5. Lobbezoo F, Ahlberg J, Raphael KG, et al. International consensus on the assessment of bruxism: Report of a work in progress. *J Oral Rehabil*. 2018;45(11):837–844. doi:10.1111/joor.12663
6. Fernandes G, Franco-Micheloni AL, Siqueira JTT, Gonçalves DAG, Camparis CM. Parafunctional habits are associated cumulatively to painful temporomandibular disorders in adolescents. *Braz Oral Res*. 2016;30:S1806-83242016000100214. doi:10.1590/1807-3107BOR-2016.vol30.0015
7. Zafar MS. Prosthodontic applications of polymethyl methacrylate (PMMA): An update. *Polymers (Basel)*. 2020;12(10):2299. doi:10.3390/polym12102299
8. Sun X, Feng Y, Jiao Y, Liu W. Fully digital workflow for the fabrication of occlusal stabilization splints based on individual mandibular movement. *J Dent*. 2024;141:104826. doi:10.1016/j.jdent.2023.104826
9. Rosentritt M, Huber C, Strasser T, Schmid A. Investigating the mechanical and optical properties of novel urethandimethacrylate (UDMA) and urethanmethacrylate (UMA) based rapid prototyping materials. *Dent Mater*. 2021;37(10):1584–1591. doi:10.1016/j.dental.2021.08.009

10. Tangpothitham S, Pongprueksa P, Inokoshi M, Mitrirattanakul S. Effect of post-polymerization with autoclaving treatment on monomer elution and mechanical properties of 3D-printing acrylic resin for splint fabrication. *J Mech Behav Biomed Mater*. 2022;126:105015. doi:10.1016/j.jmbbm.2021.105015
11. Al-Kheraif AAA. The effect of mechanical and chemical polishing techniques on the surface roughness of heat-polymerized and visible light-polymerized acrylic denture base resins. *Saudi Dent J*. 2014;26(2):56–62. doi:10.1016/j.sdentj.2013.12.007
12. Quirynen M, Bollen CM, Papaioannou W, Van Eldere J, van Steenberghe D. The influence of titanium abutment surface roughness on plaque accumulation and gingivitis: Short-term observations. *Int J Oral Maxillofac Implants*. 1996;11(2):169–178. PMID:8666447.
13. Ionescu AC, Hahnel S, König A, Brambilla E. Resin composite blocks for dental CAD/CAM applications reduce biofilm formation in vitro. *Dent Mater*. 2020;36(5):603–616. doi:10.1016/j.dental.2020.03.016
14. Astasov-Frauenhoffer M, Glauser S, Fischer J, Schmidli F, Waltimo T, Rohr N. Biofilm formation on restorative materials and resin composite cements. *Dent Mater*. 2018;34(11):1702–1709. doi:10.1016/j.dental.2018.08.300
15. Gonçalves F, Pfeifer CS, Ferracane JL, Braga RR. Contraction stress determinants in dimethacrylate composites. *J Dent Res*. 2008;87(4):367–371. doi:10.1177/154405910808700404
16. Morresi AL, D'Amario M, Capogreco M, et al. Thermal cycling for restorative materials: Does a standardized protocol exist in laboratory testing? A literature review. *J Mech Behav Biomed Mater*. 2014;29:295–308. doi:10.1016/j.jmbbm.2013.09.013
17. Eliasson ST, Tibballs J, Dahl JE. Effect of different surface treatments and adhesives on repair bond strength of resin composites after one and 12 months of storage using an improved microtensile test method. *Oper Dent*. 2014;39(5):E206–E216. doi:10.2341/12-429-L
18. Gajewski VES, Pfeifer CS, Fróes-Salgado NRG, Boaro LCC, Braga RR. Monomers used in resin composites: Degree of conversion, mechanical properties and water sorption/solubility. *Braz Dent J*. 2012;23(5):508–514. doi:10.1590/s0103-64402012000500007
19. Perea L, Matinlinna JP, Tolvanen M, Lassila LV, Vallittu PK. Fiber-reinforced composite fixed dental prostheses with various pontics. *J Adhes Dent*. 2014;16(2):161–168. doi:10.3290/j.jad.a30755
20. Murat S, Alp G, Alatalı C, Uzun M. In vitro evaluation of adhesion of *Candida albicans* on CAD/CAM PMMA-based polymers. *J Prosthodont*. 2019;28(2):e873–e879. doi:10.1111/jopr.12942
21. Steinmassl PA, Klaunzer F, Steinmassl O, Dumfahrt H, Grunert I. Evaluation of currently available CAD/CAM denture systems. *Int J Prosthodont*. 2017;30(2):116–122. doi:10.11607/ijp.5031
22. Revilla-León M, Özcan M. Additive manufacturing technologies used for processing polymers: Current status and potential application in prosthetic dentistry. *J Prosthodont*. 2019;28(2):146–158. doi:10.1111/jopr.12801
23. Tahayeri A, Morgan MC, Fugolin AP, et al. 3D printed versus conventionally cured provisional crown and bridge dental materials. *Dent Mater*. 2018;34(2):192–200. doi:10.1016/j.dental.2017.10.003
24. Revilla-León M, Meyers MJ, Zandinejad A, Özcan M. A review on chemical composition, mechanical properties, and manufacturing work flow of additively manufactured current polymers for interim dental restorations. *J Esthet Restor Dent*. 2019;31(1):51–57. doi:10.1111/jerd.12438
25. Asmussen E, Peutzfeldt A. Influence of selected components on crosslink density in polymer structures. *Eur J Oral Sci*. 2001;109(4):282–285. doi:10.1034/j.1600-0722.2001.00057.x
26. Berli C, Thieringer FM, Sharma N, et al. Comparing the mechanical properties of pressed, milled, and 3D-printed resins for occlusal devices. *J Prosthet Dent*. 2020;124(6):780–786. doi:10.1016/j.prosdent.2019.10.024
27. Digholkar S, Madhav VNV, Palaskar J. Evaluation of the flexural strength and microhardness of provisional crown and bridge materials fabricated by different methods. *J Indian Prosthodont Soc*. 2016;16(4):328–334. doi:10.4103/0972-4052.191288
28. Rayyan MM, Aboushelib M, Sayed NM, Ibrahim A, Jimbo R. Comparison of interim restorations fabricated by CAD/CAM with those fabricated manually. *J Prosthet Dent*. 2015;114(3):414–419. doi:10.1016/j.prosdent.2015.03.007
29. Meissner H, Vacquier M, Kresse-Walczak K, Boening K. Mechanical, optical and surface properties of 3D-printed and conventionally processed polyamide 12. *Dent Med Probl*. 2024;61(5):729–738. doi:10.17219/dmp/186712
30. Wieckiewicz M, Opitz V, Richter G, Boening KW. Physical properties of polyamide-12 versus PMMA denture base material. *Biomed Res Int*. 2014;2014:150298. doi:10.1155/2014/150298
31. Prpić V, Schauperl Z, Ćatić A, Dulčić N, Čimić S. Comparison of mechanical properties of 3D-printed, CAD/CAM, and conventional denture base materials. *J Prosthodont*. 2020;29(6):524–528. doi:10.1111/jopr.13175
32. Weżgowiec J, Małysa A, Więckiewicz M. How does artificial aging affect the mechanical properties of occlusal splint materials processed via various technologies? *Dent Med Probl*. 2025;62(3):527–535. doi:10.17219/dmp/174708
33. Weżgowiec J, Małysa A, Szlasa W, et al. Biocompatibility of 3D-printed vs. thermoformed and heat-cured intraoral appliances. *Front Bioeng Biotechnol*. 2024;12:1453888. doi:10.3389/fbioe.2024.1453888
34. Bociong K, Szczesio A, Sokolowski K, et al. The influence of water sorption of dental light-cured composites on shrinkage stress. *Materials (Basel)*. 2017;10(10):1142. doi:10.3390/ma10101142
35. Øilo M, Bakken V. Biofilm and dental biomaterials. *Materials (Basel)*. 2015;8(6):2887–2900. doi:10.3390/ma8062887
36. Mair LH, Stolarski TA, Vowles RW, Lloyd CH. Wear: Mechanisms, manifestations and measurement. Report of a workshop. *J Dent*. 1996;24(1–2):141–148. doi:10.1016/0300-5712(95)00043-7
37. Bollen CM, Lambrechts P, Quirynen M. Comparison of surface roughness of oral hard materials to the threshold surface roughness for bacterial plaque retention: A review of the literature. *Dent Mater*. 1997;13(4):258–269. doi:10.1016/s0109-5641(97)80038-3
38. Heimer S, Schmidlin PR, Stawarczyk B. Effect of different cleaning methods of polyetheretherketone on surface roughness and surface free energy properties. *J Appl Biomater Funct Mater*. 2016;14(3):e248–e255. doi:10.5301/jabfm.5000291
39. Wieckiewicz M, Boening KW, Richter G, Wieckiewicz W. Effect of thermocycling on the shear bond strength of different resins bonded to thermoplastic foil applied in occlusal splint therapy. *J Prosthodont*. 2015;24(3):220–224. doi:10.1111/jopr.12190
40. Gale MS, Darvell BW. Thermal cycling procedures for laboratory testing of dental restorations. *J Dent*. 1999;27(2):89–99. doi:10.1016/s0300-5712(98)00037-2
41. VDI/VDE Richtlinien. VDI/VDE 2602 Part 2. Surface Measurement. 2018. https://www.vdi.de/fileadmin/pages/vdi_de/redakteure/richtlinien/inhaltsverzeichnisse/2768785.pdf. Accessed October 21, 2025.
42. Mahross HZ, Mohamed MD, Hassan AM, Baroudi K. Effect of cigarette smoke on surface roughness of different denture base materials. *J Clin Diagn Res*. 2015;9(9):ZC39–ZC42. doi:10.7860/JCDR/2015/14580.6488
43. Szczesio-Wlodarczyk A, Domarecka M, Kopacz K, Sokolowski J, Bociong K. An evaluation of the properties of urethane dimethacrylate-based dental resins. *Materials (Basel)*. 2021;14(11):2727. doi:10.3390/ma14112727
44. Barszczewska-Rybarek IM, Krasowska M. Fractal analysis of heterogeneous polymer networks formed by photopolymerization of dental dimethacrylates. *Dent Mater*. 2012;28(6):695–702. doi:10.1016/j.dental.2012.03.015
45. N'Diaye M, Pascaretti-Grizon F, Massin P, Baslé MF, Chappard D. Water absorption of poly(methyl methacrylate) measured by vertical interference microscopy. *Langmuir*. 2012;28(31):11609–11614. doi:10.1021/la302260a
46. Giti R, Firouzmandi M, Zare Khafri N, Ansarifard E. Influence of different concentrations of titanium dioxide and copper oxide nanoparticles on water sorption and solubility of heat-cured PMMA denture base resin. *Clin Exp Dent Res*. 2022;8(1):287–293. doi:10.1002/cre2.527
47. Hemmati MA, Vafaee F, Allahbakhshi H. Water sorption and flexural strength of thermoplastic and conventional heat-polymerized acrylic resins. *J Dent (Tehran)*. 2015;12(7):478–484. PMID:26877737.
48. Saini RS, Vaddamanu SK, Dermawan D, Mosaddad SA, Heboyan A. Investigating the role of temperature and moisture on the degradation of 3D-printed polymethyl methacrylate dental materials through molecular dynamics simulations. *Sci Rep*. 2024;14(1):26079. doi:10.1038/s41598-024-77736-5

49. Ayre WN, Denyer SP, Evans SL. Ageing and moisture uptake in polymethyl methacrylate (PMMA) bone cements. *J Mech Behav Biomed Mater*. 2014;32(100):76–88. doi:10.1016/j.jmbbm.2013.12.010
50. Bettencourt AF, Neves CB, de Almeida MS, et al. Biodegradation of acrylic based resins: A review. *Dent Mater*. 2010;26(5):e171–e180. doi:10.1016/j.dental.2010.01.006
51. Neoh SP, Khantachawana A, Santiwong P, Chintavalakorn R, Srikhirin T. Effect of post-processing on the surface, optical, mechanical, and dimensional properties of 3D-printed orthodontic clear retainers. *Clin Oral Investig*. 2025;29(1):48. doi:10.1007/s00784-024-06120-4
52. Xediek Consani RL, Lacerda Vieira M, Ferraz Mesquita M, Coelho Sinhoreti MA, Danil Guiraldo R, Arruda Nóbilo MA. Effect of polymerization cycles on the linear dimensional change, hardness and impact strength of denture base acrylic resins. *Minerva Stomatol*. 2012;61(6):273–282. PMID:22669057.
53. Drotning WD, Roth EP. Effects of moisture on the thermal expansion of poly(methylmethacrylate). *J Mater Sci*. 1989;24:3137–3140. doi:10.1007/BF01139031
54. Singh S, Palaskar JN, Mittal S. Comparative evaluation of surface porosities in conventional heat polymerized acrylic resin cured by water bath and microwave energy with microwavable acrylic resin cured by microwave energy. *Contemp Clin Dent*. 2013;4(2):147–151. doi:10.4103/0976-237X.114844
55. Steinmassl PA, Wiedemair V, Huck C, et al. Do CAD/CAM dentures really release less monomer than conventional dentures? *Clin Oral Investig*. 2017;21(5):1697–1705. doi:10.1007/s00784-016-1961-6
56. Unkovskiy A, Schmidt F, Beuer F, Li P, Spintzyk S, Kraemer Fernandez P. Stereolithography vs. direct light processing for rapid manufacturing of complete denture bases: An in vitro accuracy analysis. *J Clin Med*. 2021;10(5):1070. doi:10.3390/jcm10051070
57. Alharbi N, Alharbi S, Cuijpers VMJI, Osman RB, Wismeijer D. Three-dimensional evaluation of marginal and internal fit of 3D-printed interim restorations fabricated on different finish line designs. *J Prosthodont Res*. 2018;62(2):218–226. doi:10.1016/j.jpor.2017.09.002
58. Goracci C, Bosoni C, Marti P, Scotti N, Franchi L, Vichi A. Influence of printing orientation on surface roughness and gloss of 3D printed resins for orthodontic devices. *Materials (Basel)*. 2025;18(3):523. doi:10.3390/ma18030523
59. Paradowska-Stolarz A, Wezgowiec J, Malysa A, Wieckiewicz M. Effects of polishing and artificial aging on mechanical properties of Dental LT Clear® resin. *J Funct Biomater*. 2023;14(6):295. doi:10.3390/jfb14060295
60. de Gois Moreira FG, da Silva NR, Bezerra MGPG, et al. Influence of 3D printing system, postpolymerization and aging protocols on resin flexural strength and dimensional stability for printing occlusal splints, models and temporary restorations. *Clin Oral Investig*. 2024;28(11):604. doi:10.1007/s00784-024-05998-4
61. Durkan R, Oyar P. Comparison of mechanical and dynamic mechanical behaviors of different dental resins polymerized by different polymerization techniques. *Niger J Clin Pract*. 2018;21(9):1144–1149. doi:10.4103/njcp.njcp_423_17
62. Bollen CM, Papaioanno W, Van Eldere J, Schepers E, Quirynen M, van Steenberghe D. The influence of abutment surface roughness on plaque accumulation and peri-implant mucositis. *Clin Oral Implants Res*. 1996;7(3):201–211. doi:10.1034/j.1600-0501.1996.070302.x
63. Aslanimehr M, Rezvani S, Mahmoudi A, Moosavi N. Comparison of *Candida albicans* adherence to conventional acrylic denture base materials and injection molding acrylic materials. *J Dent (Shiraz)*. 2017;18(1):61–64. PMID:28280761.
64. Paradowska-Stolarz AM, Wieckiewicz M, Mikulewicz M, et al. Comparison of the tensile modulus of three 3D-printable materials used in dentistry. *Dent Med Probl*. 2023;60(3):505–511. doi:10.17219/dmp/166070
65. Paradowska-Stolarz A, Wieckiewicz M, Kozakiewicz M, Jurczyszyn K. Mechanical properties, fractal dimension, and texture analysis of selected 3D-printed resins used in dentistry that underwent the compression test. *Polymers (Basel)*. 2023;15(7):1772. doi:10.3390/polym15071772

Original paper

Impact of self-adhesive resin cements on shear bond strength to CAD/CAM silicate ceramics

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Abstract

Background. The bond strength between self-adhesive resin cements (SARCs) and computer-aided design/computer-aided manufacturing (CAD/CAM) ceramics is crucial for restoration durability, yet data on the aging effects of different SARCs remains limited.

Objectives. The aim of the study was to evaluate the shear bond strength (SBS) and failure modes of various SARCs on 2 CAD/CAM silicate ceramics after thermal aging.

Material and methods. A total of 360 samples from 2 ceramics (CEREC Tessera™ HT (CTS group); IPS Empress CAD LT (IEC group)) were treated with hydrofluoric acid (HF) and bonded with 6 SARCs: G-CEM ONE™ (GCO); Maxcem Elite™ (MXC); Nexus™ Universal (NXU); SpeedCEM® Plus (SPC); RelyX™ Universal (RLX); and PANAVIA™ SA Cement Universal (PSA). The samples underwent water storage (24 h, 37°C) or thermal aging (30 days, 20,000 cycles, 5–55°C). The shear bond strength and failure modes were measured, with the bonding interfaces being assessed via scanning electron microscopy (SEM). A multifactorial analysis of variance (ANOVA) was applied for the statistical analysis.

Results. Significant differences were identified in aging ($F = 117.64$, $p < 0.001$), ceramic types ($F = 28.91$, $p < 0.001$) and among SARCs ($F = 34.79$, $p < 0.001$). The highest SBS post-aging was found with IEC+GCO (24.92 ±2.90 MPa) and CTS+MXC (21.68 ±3.16 MPa), while the lowest SBS was recorded with CTS+PSA (6.22 ±4.31 MPa). Failure modes shifted from cohesive to mixed after thermocycling.

Conclusions. All tested SARCs bond effectively to CAD/CAM ceramics, with GCO and PSA being recommended for IEC ceramics, and MXC for CTS ceramics to optimize bond strength.

Keywords: CAD/CAM, resin cements, aging, bond strength, glass ceramics

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Highlights

- Self-adhesive resin cements (SARCs) achieve clinically acceptable bond strength to CAD/CAM silicate ceramics, but their performance is strongly material-dependent.
- Leucite-reinforced ceramics (IPS Empress CAD LT (IEC)) exhibit higher and more stable bond strength than advanced lithium disilicate ceramics (CEREC Tessera™ HT (CTS)).
- Cement–ceramic compatibility is critical: G-CEM ONE™ and PANAVIA™ SA Cement Universal perform best with IEC, whereas Maxcem Elite™ shows the most reliable bonding to CTS.
- Thermocycling significantly reduces bond strength across all SARCs and ceramic types, indicating compromised long-term durability.
- Aging promotes a shift from predominantly cohesive failures to mixed and adhesive failures, reflecting progressive interfacial degradation.

Introduction

Modern computer-aided design/computer-aided manufacturing (CAD/CAM) technologies utilize highly advanced silicate ceramics, valued for their superior aesthetics and mechanical properties, which have led to their widespread adoption in restorations such as veneers, crowns, inlays, and onlays.[1,2] These ceramics incorporate crystals like feldspar, leucite, lithium disilicate, or zirconia, which, combined with heat and pressure, create homogeneity and reduce microcracks. The CAD/CAM processes further streamline restorations.[3]

Adhesive bonding is the preferred method for silicate ceramic restorations, often employing hydrofluoric acid (HF) etching[4,5] followed by silanization.[6,7] Hydrofluoric acid etching selectively dissolves the glassy phase of the ceramic, creating a micro-retentive surface that enhances micromechanical interlocking, while silanization promotes chemical bonding between the ceramic and resin-based adhesives. Alternative surface treatments, such as air particle abrasion with aluminum oxide particles (Al_2O_3), silica-coated aluminum oxide, diamond bur grinding and laser irradiation, can result in comparable bond strength when combined with an adhesive.[8,9] Akar et al. demonstrated that neodymium-doped yttrium aluminium garnet (Nd:YAG) laser irradiation (1–3 W) significantly enhanced the bond strength of self-adhesive resin cements (SARCs) to pre-sintered zirconia, while airborne particle abrasion may reduce it.[10] The use of SARCs is increasing among general dentists and practitioners, as they simplify the process by eliminating the need for silane application and are often used in combination with universal adhesives.

The quality of adhesion depends on the bonded surface, surface treatment, ceramic material, adhesive and cement composition, and the amount of enamel or dentin present.[4] Achieving stable restorations and reduced fracture risk is possible through physicochemical interactions at the interface between the tooth, resin cement and ceramic, enabling efficient stress transfer and enhancing restoration durability.[11]

Bonding agents range from multi-step composite cements to SARCs, which simplify clinical application by combining conventional and adhesive properties.[12] With a low film thickness, high retention and mechanical strength, SARCs are ideal for non-metal-supported ceramics and offer a user-friendly, single-step application.[13–15] Dual-curing SARCs can be self- or photo-activated, eliminating the need for separate pre-treatments or bonding agents.[14] Their bond strength rivals that of traditional composite cements, though polymerization inefficiency in SARCs may impact adhesion durability.[16]

A recent study suggests that PANAVIA™ V5 achieves better results with certain ceramics, while SARCs demonstrate weaker outcomes.[17] Despite extensive research on adhesive bonding in CAD/CAM restorations, current findings reveal considerable variability in bond strength outcomes, particularly for SARCs when applied to lithium disilicate ceramics.[18] Additionally, thermocycling, a widely used aging protocol, has inconsistent effects on bonding durability across studies, complicating the interpretation of results and limiting clinical recommendations.[19] These inconsistencies highlight critical knowledge gaps, especially regarding the performance of newly marketed SARCs and their interaction with different CAD/CAM ceramic systems.

While CAD/CAM restorations generally demonstrate favorable outcomes, they are prone to marginal decline over time due to composite wear and bond loss.[5,20] Debonding, especially with light-cured materials, often results from incomplete polymerization at the dentin interface.[5] The use of dual-curing materials or adhesive layers can enhance bonding and reduce failure risk,[21] although incomplete polymerization may release unreacted monomers, leading to hygroscopic swelling, cracks and restoration failure.[22,23]

The effectiveness of SARCs and additional surface treatments remains the subject of debate.[24] While some

studies advocate for extra surface treatments to optimize bonding, others find SARCs effective as standalone solutions.[12] However, inconsistencies in bonding performance, particularly under simulated intraoral aging conditions, highlight the need for systematic investigation. A significant challenge lies in the lack of standardized protocols for artificial aging, which contributes to variability and limits comparability in bonding studies.[15] Inconsistencies in bonding data for self-adhesives and CAD/CAM ceramics, particularly lithium disilicate ceramics, underline the need for systematic investigation.

Self-adhesive resin cements offer clinical advantages, such as simplified application and bond strength comparable to multi-step systems, but their long-term performance remains insufficiently characterized. Addressing these gaps is essential to fully understand their interaction with different CAD/CAM ceramic systems and to provide robust clinical recommendations.

The aim of the present study is to test the latest SARCs and their interaction with CAD/CAM silicate ceramics under thermocycling, evaluating both their bond strength and long-term stability. Additionally, the study's objective is to compare multiple novel SARCs with distinct monomer compositions, filler technologies and polymerization characteristics, focusing on shear bond strength (SBS) after water storage and thermocycling. By assessing their potential to improve adhesion without the need for separate silane pretreatment, we aim to provide clinically relevant insights into their durability and effectiveness. The null hypotheses tested were: (1) SARC type does not affect bond strength to CAD/CAM ceramics; (2) SARC type does not influence the failure mode.

Material and methods

Sample size estimation

A priori sample size estimation was performed using G*Power v. 3.1 software (Heinrich Heine University, Düsseldorf, Germany) to achieve a power of 80% ($\alpha = 0.05$) for the multifactorial analysis of variance (ANOVA). The calculation of the total sample size was based on a medium effect size ($f = 0.25$) and 24 subgroups, with the resultant sample size of 270 specimens being distributed evenly across the subgroups. However, to enhance statistical power and ensure more robust results, a total of 360 specimens were ultimately used.

Preparation of study specimens

A total of 360 ceramic samples were prepared from CAD/CAM advanced lithium disilicate ceramic blocks (CEREC Tessera™ HT; Dentsply Sirona, Bensheim, Germany) (CTS) and CAD/CAM leucite-reinforced silicate ceramic blocks (IPS Empress CAD LT; Ivoclar Vivadent, Schaan, Liechtenstein) (IEC) according to the study protocol (Fig. 1). The blocks were sectioned into 15-mm diameter discs with a thickness of 2.2 mm using a diamond saw under water cooling (Secotom-50; Struers, Ballerup, Denmark; and IsoMet® 1000; Buehler, Lake Bluff, USA). The dimensions of the samples were verified with a digital caliper (Alpha Professional Tools, Franklin, USA), and all surfaces were standardized through sequential grinding from P320 to P1200 silicon carbide sandpapers (SiC Foil; Struers). A total of 180 samples per ceramic type were produced and divided into 24 subgroups of 15 specimens each. Table 1 summarizes the materials used, including their brand names, manufacturers and chemical compositions. All ceramic samples were etched with 9% buffered HF (Ultradent™ Porcelain Etch; Ultradent Products, South Jordan, USA), following the manufacturer's recommendations (CTS: 30 s; IEC: 60 s). Thereafter, the samples were cleaned in an ultrasonic bath with distilled water for 8 min, and air-dried.

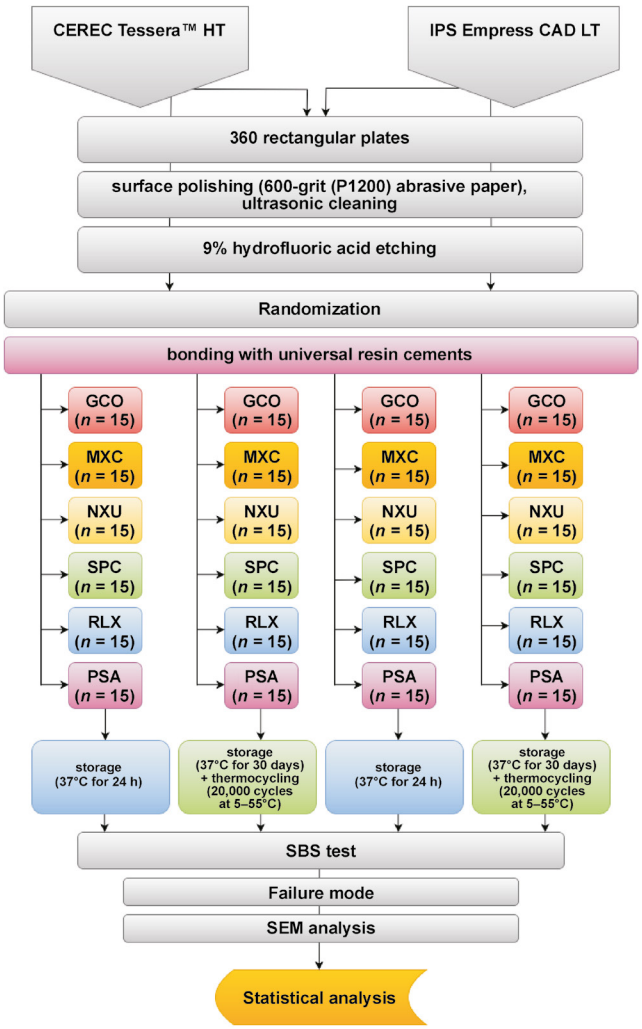

Fig. 1. Flowchart of the study

GCO – G-CEM ONE™; MXC – Maxcem Elite™; NXU – Nexus™ Universal; SPC – SpeedCEM® Plus; RLX – RelyX™ Universal; PSA – PANAVIA™ SA Cement Universal; SBS – shear bond strength; SEM – scanning electron microscopy.

Table 1. Characteristics of the materials used in the study

Brand/manufacturer	Abbreviation	Batch No.	Chemical composition
IPS Empress CAD LT; Ivoclar Vivadent, Schaan, Liechtenstein	IEC	YB54PB	SiO_2 (60.0–65.0%) Al_2O_3 (16.0–20.0%) K_2O (10.0–14.0%) Na_2O (3.5–6.5%) CaO other oxides pigments
CEREC Tessera™ HT; Dentsply Sirona, Bensheim, Germany	CTS	16015667	$Li_2O_5Si_2$ (90.0%) Li_3PO_4 (5.0%) $LiAlSi_2O_6$ (virgilite) (5.0%)
Ultradent™ Porcelain Etch; Ultradent Products, South Jordan, USA	–	BW3LM	9% hydrofluoric acid
G-CEM ONE™; GC Corporation, Tokyo, Japan	GCO	2312051	7,7,9-trimethyl-4,13-dioxo-3,14-dioxa-5,12-diazahexadecane-1,16-diyl bis(methacrylate) 2-hydroxy-1,3-dimethacryloxypropane 10-methacryloyloxydecyl dihydrogen phosphate α,α-dimethylbenzyl hydroperoxide 6-tert-butyl-2,4-xylenol
Maxcem Elite™; Kerr Corporation, Orange, USA	MXC	10463885	2-hydroxyethyl methacrylate 4-methoxyphenol cumene hydroperoxide α,α-dimethylbenzyl hydroperoxide methacrylate ester monomer titanium dioxide pigment
Nexus™ Universal; Kerr Corporation, Orange, USA	NXU	8024703	(1-methylethylidene)bis[4,1-phenyleneoxy(2-hydroxy-3,1-propandiyl)]bismethacrylate 2-hydroxy-1,3-propandiyl bismethacrylate 2-hydroxyethyl methacrylate silanamine 1,1,1-trimethyl-N-(trimethylsilyl) 2,2′-ethylenedioxydiethyl dimethacrylate ytterbium(III) fluoride propyldynetrimethanol ethoxylated esters with acrylic acid 7,7,9-trimethyl-4,13-dioxo-3,14-dioxa-5,12-diaz hexadecan-1,16-diyl-bis(methacrylate)
SpeedCEM® Plus; Ivoclar Vivadent, Schaan, Liechtenstein	SPC	Z06T2Z	urethane dimethacryclate triethylene glycol dimethacrylate ytterbium(III) fluoride methacrylate phosphoric acid ester dibenzoyl peroxide 1,10-decanediol dimethacrylate polyethylene glycol dimethacrylate
RelyX™ Universal; 3M Deutschland GmbH, Neuss, Germany	RLX	10896493	urethane dimethacrylate ytterbium(III) fluoride glass powder (65997-17-3) surface treated with 2-propenoic acid, 2-methyl-,3-(trimethoxysilyl) propyl ester (2530-85-0) and trimethoxyphenylsilane (2996-92-1) bulk material triethylene glycol dimethacrylate L-ascorbic acid 6-hexadecanoate hydrate (1:2) triethoxy(octyl)silane hydrolysis products with silica 2-hydroxyethyl methacrylate titanium dioxide triphenyl phosphite
PANAVIA™ SA Cement Universal; Kuraray Noritake Dental Inc., Tokyo, Japan	PSA	430316	bisphenol A diglycidyl methacrylate triethylene glycol dimethacrylate 2-hydroxyethyl methacrylate sodium fluoride titanium dioxide dipotassium peroxodisulfate

The samples were bonded with 6 SARCs: G-CEM ONE™ (GC Corporation, Tokyo, Japan) (GCO); Maxcem Elite™ (Kerr Corporation, Orange, USA) (MXC); Nexus™ Universal (Kerr Corporation) (NXU); SpeedCEM® Plus (Ivoclar Vivadent) (SPC); RelyX™ Universal (3M Deutschland GmbH, Neuss, Germany) (RLX); and PANAVIA™ SA Cement Universal (Kuraray Noritake Dental Inc., Tokyo, Japan) (PSA). The luting composite (SARC) was preheated in an incubator at 37°C for 1 min before application. Each ceramic specimen was positioned in a silicone mold to ensure a standardized bonding area. A Teflon mold (5 mm × 2 mm) was placed on the ceramic surface to define the shape of the composite cylinder, in accordance with ISO 10477.[25] The composite material was applied in 2 increments of 1 mm each, with each layer being light-cured for 20 s using a light-emitting diode (LED) curing unit (Bluephase® Style; Ivoclar Vivadent) at 1,200 mW/cm^2. After the removal of the Teflon mold, an additional 20-second light-curing step was performed to ensure complete polymerization (Fig. 2). All procedures were performed by the same operator (MJ) to ensure consistency.

Aging protocol

Half of the specimens were stored in distilled water for 24 h at 37°C (non-aged group: NAG), while the other half underwent aging (aged group: AG) by storing in distilled water for 30 days at 37°C, followed by 20,000 thermocycles (5–55°C) with 30-second dwell time and 5-second transfer time (LAUDA RC 20 CS; Lauda, Lauda-Königshofen, Germany).

Evaluation of adhesion performance

After bonding, a 2-hour resting period was allowed. Then, the SBS measurements, expressed in megapascals (MPa), were performed using a universal testing device (zwickiLine Z0.5 TN; ZwickRoell, Ulm, Germany) at a crosshead speed of 0.5 mm/min. These metrics were calculated by dividing the breaking load, recorded in Newtons (N), by the bonding area, which was 19.63 mm^2 for each sample. During the testing phase, the bonded surface was aligned with the force application mechanism. Shear forces were applied at the composite–ceramic interface using a knife-edge indenter, positioned to closely approximate the interface, ensuring precise measurement of bonding efficacy (Fig. 3).

Assessment of fracture patterns

Fracture modes were analyzed under a stereomicroscope (VHX-5000; Keyence Corp., Osaka, Japan) at ×40 magnification. Two independent evaluators (CS, MJ) classified failures as adhesive (at the composite–ceramic interface), cohesive (within the ceramic) or mixed (combining both types).

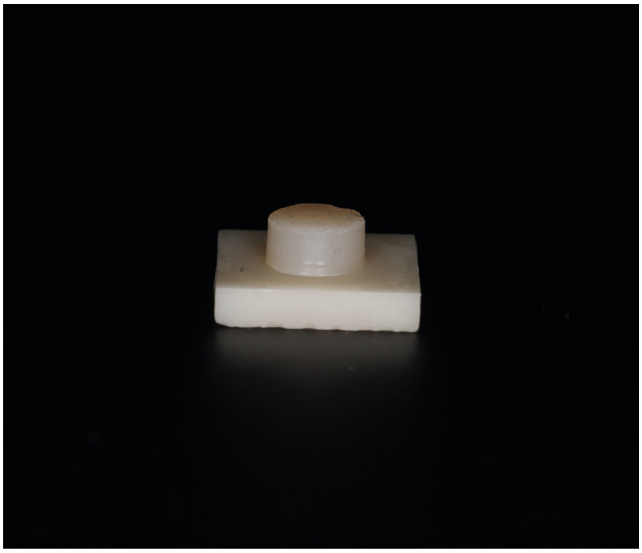

Fig. 2. Ceramic specimen with bonded composite

Analysis of adhesive interface using SEM

One specimen per group was randomly selected for scanning electron microscopy (SEM) analysis of the adhesive interface. The samples were coated with a conductive gold-palladium layer (Q150T Plus; Quorum, Laughton, UK) and imaged at ×1,000 magnification (1,536 px × 1,024 px) using a scanning electron microscope (Sigma 360 VP; Carl Zeiss, Oberkochen, Germany).

Statistical analysis

The statistical analysis was conducted using the IBM SPSS Statistics for Windows software, v. 24.0 (IBM Corp., Armonk, USA). The data was normally distributed (Shapiro–Wilk test), and homogeneity of variances was confirmed. A multifactorial ANOVA with Bonferroni correction was used to evaluate differences in bond strength among ceramics and cements, with the significance level set at $\alpha < 0.05$.

Fig. 3. Shear bond strength (SBS) testing setup using a universal testing machine (zwickiLine Z0.5 TN; ZwickRoell, Ulm, Germany) with a bonded ceramic–composite specimen

Results

The assumptions for multifactorial analysis of variance were verified. The normal distribution of the data, assessed using the Shapiro–Wilk test, and the homogeneity of error variances, evaluated with the Levene's test, were confirmed at both time points ($p < 0.001$). Aging induced by thermocycling led to a significant reduction in SBS values across all groups. There were notable differences between the pre- and post-thermocycling time points ($F = 117.64$, degrees of freedom (df) = 1, $p < 0.001$, $\varepsilon^2 = 0.205$). Additionally, significant differences were observed between the 2 ceramics examined ($F = 28.91$, $df = 1$, $p < 0.001$, $\varepsilon^2 = 0.060$), as well as among the universal cements ($F = 34.79$, $df = 5$, $p < 0.001$, $\varepsilon^2 = 0.276$). A significant interaction effect between the combination of ceramic type, universal cement and aging was also demonstrated ($F = 10.99$, $df = 5$, $p < 0.001$, $\varepsilon^2 = 0.108$).

Table 2 presents the SBS values for each group. The data indicated that the aged groups exhibited significantly lower values than those subjected to only 24 h of water storage. Prior to thermocycling, the highest SBS values were observed in the IEC group for PSA (26.02 ±2.28 MPa) and GCO (25.61 ±4.54 MPa), while in the CTS group, GCO (27.25 ±5.95 MPa) and MXC (23.26 ±2.50 MPa) were leading. After thermocycling, GCO was identified as the most robust material in the IEC group (24.92 ±2.90 MPa), followed by PSA (21.64 ±2.98 MPa). For CTS, MXC demonstrated the highest SBS values after aging (21.68 ±3.16 MPa). The lowest SBS after thermocycling was recorded for RLX in the IEC group (14.91 ±4.35 MPa) and for PSA in the CTS group (6.22 ±4.31 MPa). The significant differences between the various combinations of SARCs and ceramics, as well as pairwise comparisons for SBS values, are presented in Table 2.

The failure mode analysis (Fig. 4) showed that before thermocycling, the failures were cohesive, in both the IEC and CTS groups. After thermocycling, an increase in adhesive and mixed failure types was observed, with adhesive failures occurring sporadically. Representative SEM

Table 2. Shear bond strength (SBS) of 6 self-adhesive resin cements (SARCs) bonded to 2 ceramic substrates, measured under non-aged and aged conditions

Resin cement	IEC		CTS	
	non-aged	aged	non-aged	aged
GCO	25.61 ±4.54	24.92 ±2.90	27.25 ±5.95	19.91 ±4.22^{A}
MXC	21.76 ±3.18^{a}	15.72 ±6.10^{aB}	23.26 ±2.50	21.68 ±3.16^{aB}
NXU	20.68 ±4.54^{ab}	19.30 ±5.43^{a}	16.14 ±6.27^{ab}	16.17 ±5.47^{ab}
SPC	21.04 ±1.59^{abc}	20.79 ±3.96^{ab}	22.49 ±3.12^{bc}	14.75 ±3.99^{abcD}
RLX	16.30 ±73.72^{abd}	14.91 ±4.35^{abd}	16.93 ±1.33^{a}	18.68 ±3.47^{abd}
PSA	26.02 ±2.28^{abcde}	21.64 ±2.98^{abcde}	21.54 ±0.96^{abcde}	6.22 ±4.31^{abcdeD}
Total	21.91 ±4.72	19.55 ±5.55	21.27 ±5.42	16.23 ±6.49

Different lowercase letters indicate statistically significant differences among resin cements, while capital letters represent statistically significant differences within each ceramic group between aging conditions ($p < 0.05$, ANOVA).

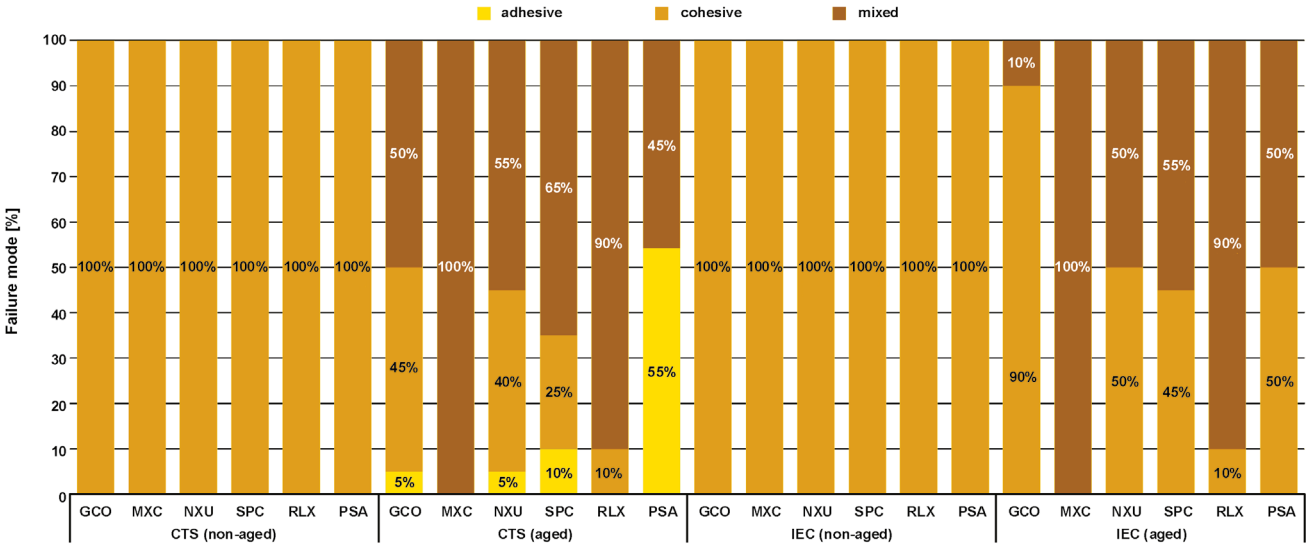

Fig. 4. Distribution of failure modes for self-adhesive resin cements (SARCs) bonded to 2 ceramic types, evaluated after 24 h of storage without aging and after aging by 20,000 thermocycles

CTS – CEREC Tessera™ HT; IEC – IPS Empress CAD LT.

images of the bonding interfaces from each group (AG and NAG) are shown in Fig. 5 and Fig. 6 at ×1,000 magnification. The images predominantly revealed continuous bonding zones. In the aged groups, more gaps and discontinuities were observed, with the resin showing fraying and the resin cement appearing looser. The bonding layers exhibited varying thicknesses and irregular structures, and the continuity of the adhesive layer was inconsistent. While top-down images suggested the presence of resin tags as surface features, their full extent and depth could not be assessed. Distinct differences in the microstructures of ceramics' surfaces were revealed. The IEC group exhibited more porous and loosened structures after etching, with noticeable microporosities (Fig. 5). The interface between the ceramic and the composite appeared poorly defined and displayed numerous cracks. In contrast, the CTS group presented smoother etched surfaces, with small cracks and irregularities dispersed throughout the material (Fig. 6).

Discussion

The study aimed to investigate the impact of SARCs on the bond strength to 2 different CAD/CAM silicate ceramics (CTS and IEC) under various durability conditions. The results indicated that the bond strength remained consistent after thermocycling, with SBS values exhibiting only

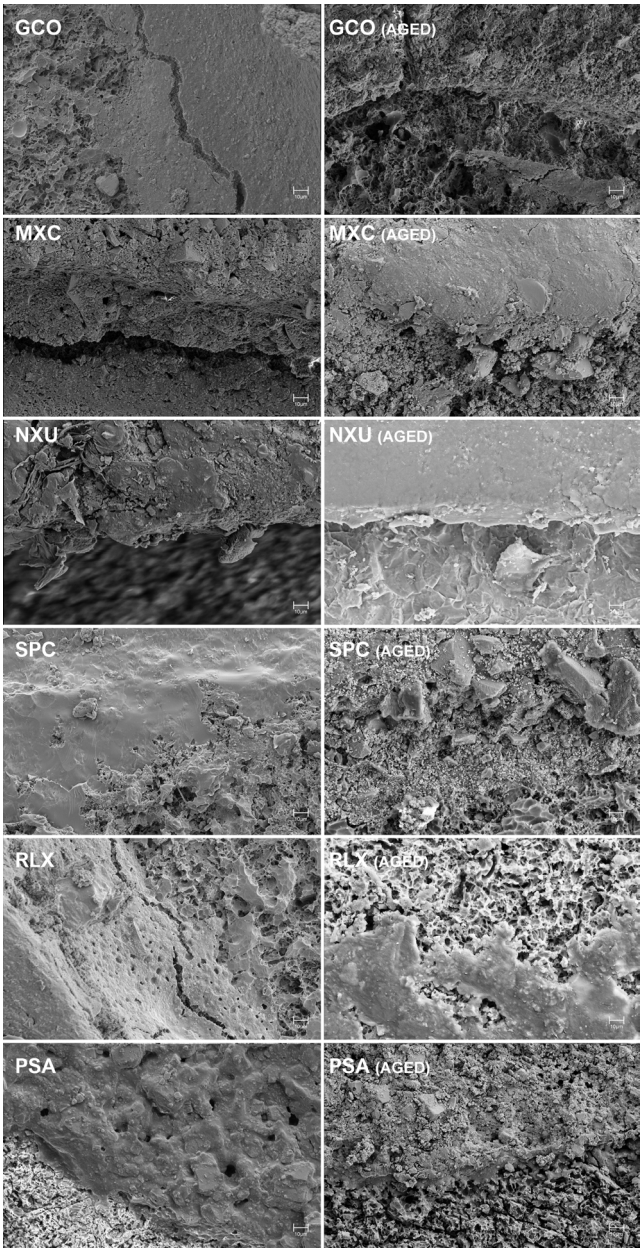

Fig. 5. Scanning electron microscopy (SEM) images (×1,000 magnification) of the interface between IPS Empress CAD LT (IEC) ceramic and self-adhesive resin cements (SARCs) after 24 h without aging and after aging by 20,000 thermocycles

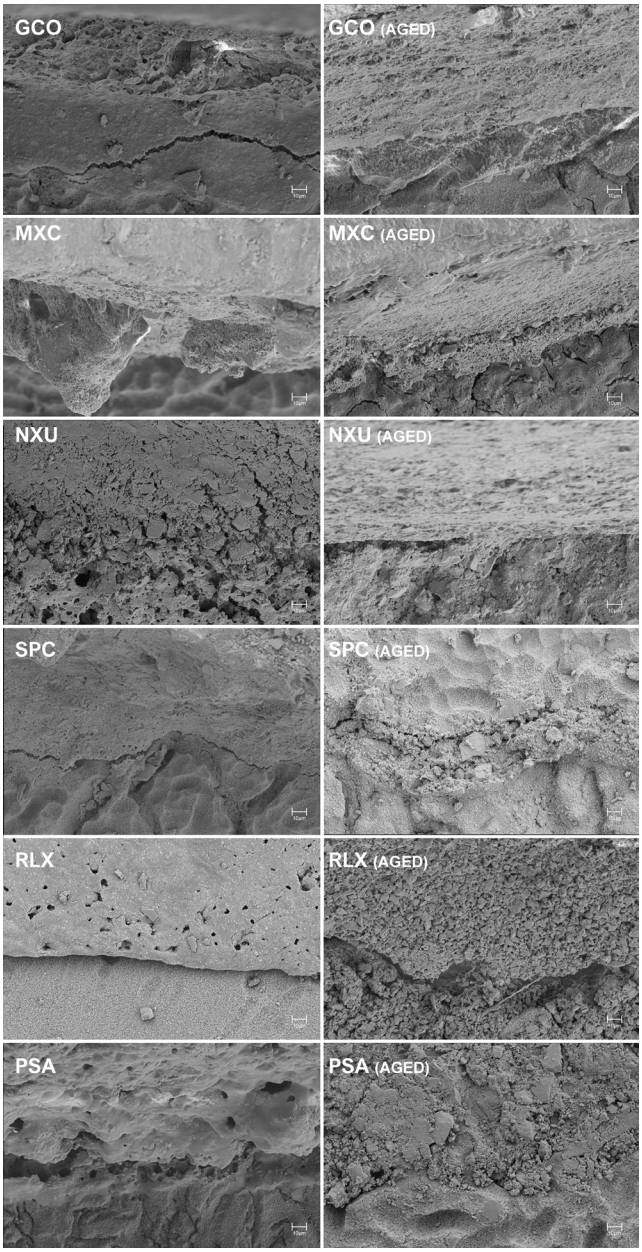

Fig. 6. Scanning electron microscopy (SEM) images (×1,000 magnification) of the interface between CEREC Tessera™ HT (CTS) ceramic and self-adhesive resin cements (SARCs) after 24 h without aging and after aging by 20,000 thermocycles

a slight decrease compared to 24-h water storage. However, the observed variations depended on the ceramic microstructure, particularly the differing compositions of the 2 tested glass ceramics, as well as on hydrolytic degradation and the specific composition of the resin cement. The novel SARCs used in this study are characterized by advanced monomer systems, such as 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) and 4-methacryloxyethyltrimellitic acid (4-MET), which improve bonding to ceramics without necessitating the separate application of silane. These innovations enhance polymerization efficiency, moisture resistance and bond durability. The unique features of these materials influence their adhesive performance, with further variations observed based on filler content, polymerization efficiency and hydrophilicity. However, prolonged water storage and thermal cycling caused a decrease in bond strength across all tested groups. Consequently, the null hypotheses were rejected, as it was demonstrated that SARC type significantly affected both SBS and failure modes.

Self-adhesive cements

The current study investigated a broad selection of SARCs to ensure representative coverage of various product categories. While some SARCs have been evaluated in other studies, these evaluations often occurred in isolation, limiting the direct comparability of results. The included SARCs were chosen based on their clinical relevance, market availability and unique formulation characteristics. This ensures a representative analysis of chemical compositions, polymerization mechanisms and their specific indications for CAD/CAM glass ceramics, as highlighted by manufacturers. The study aimed to provide evidence-based insights into the performance of currently marketed SARCs under standardized conditions, enabling more reliable comparisons and informed recommendations for clinical practice.

Bond strength

A major drawback of SARCs is their poor wettability, attributed to their high viscosity, which leads to limited infiltration.[26] Compared to total-etch or self-etch systems, self-adhesive cements often exhibit lower bond strength, as they may not fully eliminate the smear layer.[26,27] This can result in a weaker hybrid layer between the cement and substrate, and negatively affect the adhesion between ceramics and SARCs.[27] Furthermore, their bond strength depends not only on their viscosity and resulting wettability but also on the restorative material used. The results of this study are consistent with recent data demonstrating that SBS of cements is significantly influenced by the substrate.[28] Therefore, it is crucial to carefully match the cement to the specific substrate. The chemical composition of SARCs, particularly the functional monomers they contain, may promote a particular affinity for certain ceramic core materials. The obtained bond values were similar to the recommended SBS of 15–20 MPa between the adhesive and dentin. In this study, IEC, which requires a longer etching time, demonstrated a significantly higher bond strength compared to CTS, thereby highlighting the impact of microstructural modifications through HF. Different microstructures of IEC and CTS can be attributed to their compositions (Table 1).[29]

In this study, the interaction between CTS ceramics and respective SARCs provided adequate adhesion, with all bond values initially exceeding the minimum threshold of 5 MPa (DIN EN ISO 10477).[30] However, after aging, the bond strength of the CTS+PSA combination significantly decreased to 6.22 ±4.31 MPa, which was notably lower compared to other groups. Despite meeting the initial threshold, the significant reduction in bond strength for the CTS+PSA combination highlights that it is more susceptible to degradation over time. This decrease can be attributed to the composition of PSA cement, which contains bisphenol A diglycidyl methacrylate, triethylene glycol dimethacrylate and titanium dioxide. In combination with the CTS ceramics' virgilite and phosphate content, these components likely resulted in suboptimal chemical interaction and compromised long-term adhesion. In contrast, IEC ceramics demonstrated superior performance, particularly in conjunction with GCO cement. The IEC+GCO combination achieved the highest bond strength after aging (24.92 ±2.90 MPa), which can be attributed to the interaction between GCO cement's 10-MDP monomer and the silicon dioxide and aluminum oxide-rich reactive surface of the IEC ceramic. This reactive surface enhances adhesion, allowing IEC ceramics to consistently outperform CTS ceramics after aging. While CTS ceramics initially formed stronger bonds with certain cements, their lower reactivity compromised their long-term bonding potential. In addition, higher filler content in some cements could reduce bond strength by impeding the penetration of the adhesive resin.[27]

Thermocycling

The specimens were subjected to an aging process involving either 24-h or 30-day storage in deionized water at 37 ±1°C. This storage duration is described in DIN EN ISO 10477, and the duration of thermocycling varies between 0 and 20,000 cycles in comparable studies, with 20,000 cycles simulating approx. 2 years of clinical use.[31,32] Composites continuously absorb water in a moist environment, with water molecules diffusing through the plastic material as a permeable medium.[33] This leads to hydrolysis at the interfaces between the composite and ceramic, as well as within the composite itself, which is a primary cause of bond weakening.[34] In our study, thermocycling significantly reduced bond strength across all groups. The repeated thermal expansion and contraction

likely induced mechanical stress at the composite–ceramic interface, contributing to debonding. Additionally, the absorption of water by the composites may have accelerated the process of hydrolysis, further compromising the integrity of the bond. The findings demonstrate that the combination of thermal fatigue and hydrolytic degradation plays a critical role in the long-term durability of ceramic bonding systems under conditions mimicking the oral environment.

Fracture modes

The observed bond weakening had an influence on the fracture patterns. The IEC and CTS groups primarily exhibited cohesive failures before thermocycling, with an increase in mixed failures afterward. The CTS+PSA group displayed a significant number of adhesive fractures and the lowest bond strength. In the remaining groups, adhesive fractures occurred in around 5–10% of cases, with mixed and cohesive failures being more prevalent. These fracture patterns likely result from the uneven distribution of lithium disilicate (IEC) and virgilite crystals (CTS) combined with hydrolytic degradation. The presence of mixed failures indicates a combination of strong and weak adhesion, resulting from material heterogeneity. Thermocycling reduced bond strength across all SARCs, a finding that is consistent with the results of previous studies.[8,9]

SEM

The SEM analysis in the present study underscores the impact of aging on the adhesive interface between ceramics and SARCs. After thermocycling, the presence of gaps and resin fraying indicated compromised adhesive integrity, aligning with reduced bond strength values. The more porous, loosened structure of IEC after etching facilitated enhanced micromechanical interlocking, which correlated with higher pre-aging bond strength. However, after thermocycling, an increase in mixed failures was observed. This suggests that while the porous nature of IEC might enhance initial bonding, it may also contribute to mechanical stress and crack formation over time, leading to complex failure patterns rather than purely adhesive failures.

Despite these observations, the images indicated strong micromechanical interlocking at the composite–ceramic interface, highlighting generally robust bonding. However, the presence of gaps and structural irregularities in the aged specimens suggests potential vulnerabilities.

In contrast, CTS exhibited a smoother etched surface with fewer cracks and irregularities, which likely contributed to a weaker adhesive interface. The lower degree of porosity observed in CTS might explain the generally lower bond strength exhibited by this ceramic, as less mechanical interlocking occurred. This reduced interlocking potential may have contributed to the higher percentage of adhesive failures observed in the CTS+PSA group after aging, where the weakest bond strength was recorded.

Limitations

The SEM findings indicate potential weaknesses in the adhesive interface after thermocycling, though these observations must be interpreted within the limitations of this in vitro study. A key limitation is the inability to fully replicate intraoral conditions. Factors like temperature fluctuations, saliva and masticatory stresses are only partially simulated, limiting the direct clinical applicability of the results. Additionally, long-term effects, particularly material aging, were not fully captured within the study's timeframe. The study focused solely on the ceramic–cement bond, excluding dental hard tissue, which is essential for clinical adhesion. It is important to note that the results are limited to the adhesion between the tested ceramics and SARCs, and do not provide data on adhesion to enamel or dentin. While in vitro studies predominate due to the challenges of in vivo testing, comparing results across studies requires caution, as variations in protocols and testing conditions, along with the lack of a uniform protocol for artificial aging, complicate the comparability of study outcomes. Thus, the findings of this study are restricted to the specific ceramics tested (leucite-reinforced and lithium disilicate ceramics) and cannot be generalized to other ceramic types or clinical scenarios involving enamel or dentin. It is recommended that future research include long-term clinical evaluations, with particular emphasis on material aging and bond durability under real oral conditions.

Conclusions

All tested SARCs are suitable for bonding CAD/CAM silicate ceramics in clinical practice. However, to achieve optimal bond strength, specific recommendations should be followed based on the type of ceramic. GCO and PSA cements are the most effective for bonding leucite-reinforced silicate ceramics (IEC), providing the highest bond strength and durability. For CAD/CAM advanced lithium disilicate ceramics (CTS), MXC cement is recommended, as it demonstrated superior performance compared with the other cements. However, the process of aging and the storage weaken the adhesive bond, particularly after thermocycling, which compromises long-term durability. These findings, however, are limited to adhesive performance on the 2 tested ceramics and cannot be extrapolated to clinical conditions involving enamel or dentin adhesion.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Sjögren G, Molin M, van Dijken JWV. A 10-year prospective evaluation of CAD/CAM-manufactured (Cerec) ceramic inlays cemented with a chemically cured or dual-cured resin composite. *Int J Prosthodont*. 2004;17(2):241–246. PMID:15119879.
2. Frankenberger R, Krämer N, Appelt A, Lohbauer U, Naumann M, Roggendorf MJ. Chairside vs. labside ceramic inlays: Effect of temporary restoration and adhesive luting on enamel cracks and marginal integrity. *Dent Mater*. 2011;27(9):892–898. doi:10.1016/j.dental.2011.05.007
3. da Silva LHD, de Lima E, Miranda RBP, Favero SS, Lohbauer U, Cesar PF. Dental ceramics: A review of new materials and processing methods. *Braz Oral Res*. 2017;31(Suppl 1):e58. doi:10.1590/1807-3107BOR-2017.vol31.0058
4. Calheiros-Lobo MJ, Vieira T, Carbas R, da Silva LFM, Pinho T. Effectiveness of self-adhesive resin luting cement in CAD–CAM blocks – a systematic review and meta-analysis. *Materials (Basel)*. 2023;16(8):2996. doi:10.3390/ma16082996
5. Frankenberger R, Hartmann VE, Krech M, et al. Adhesive luting of new CAD/CAM materials. *Int J Comput Dent*. 2015;18(1):9–20. PMID:25911826.
6. Tian T, Tsoi JKH, Matinlinna JP, Burrow MF. Aspects of bonding between resin luting cements and glass ceramic materials. *Dent Mater*. 2014;30(7):e147–e162. doi:10.1016/j.dental.2014.01.017
7. Kalavacharla VK, Lawson NC, Ramp LC, Burgess JO. Influence of etching protocol and silane treatment with a universal adhesive on lithium disilicate bond strength. *Oper Dent*. 2015;40(4):372–378. doi:10.2341/14-116-L
8. Matinlinna JP, Lung CYK, Tsoi JKH. Silane adhesion mechanism in dental applications and surface treatments: A review. *Dent Mater*. 2018;34(1):13–28. doi:10.1016/j.dental.2017.09.002
9. Lima RBW, Muniz IAF, Campos DES, et al. Effect of universal adhesives and self-etch ceramic primers on bond strength to glass-ceramics: A systematic review and meta-analysis of in vitro studies. *J Prosthet Dent*. 2024;131(3):392–402. doi:10.1016/j.prosdent.2022.01.011
10. Akar T, Dündar A, Kırmalı Ö, et al. Evaluation of the shear bond strength of zirconia to a self-adhesive resin cement after different surface treatment. *Dent Med Probl*. 2021;58(4):463–472. doi:10.17219/dmp/135652
11. Ling L, Ma Y, Chen Y, Malyala R. Physical, mechanical, and adhesive properties of novel self-adhesive resin cement. *Int J Dent*. 2022;2022:4475394. doi:10.1155/2022/4475394
12. Melo Freire CA, Borges GA, Caldas D, Santos RS, Ignácio SA, Mazur RF. Marginal adaptation and quality of interfaces in lithium disilicate crowns – influence of manufacturing and cementation techniques. *Oper Dent*. 2017;42(2):185–195. doi:10.2341/15-288-L
13. De Munck J, Vargas M, Van Landuyt K, Hikita K, Lambrechts P, Van Meerbeek B. Bonding of an auto-adhesive luting material to enamel and dentin. *Dent Mater*. 2004;20(10):963–971. doi:10.1016/j.dental.2004.03.002
14. Alvarenga M, Machado L, Prado A, Veloso S, Monteiro G. Self-adhesive resin cement versus conventional cements on the failure rate of indirect single-tooth restorations: A systematic review and meta-analysis of randomized clinical trials. *J Prosthet Dent*. 2024;132(5):880.e1–880.e8. doi:10.1016/j.prosdent.2024.04.027
15. Andrews EK, Gedge JL, Vandewalle KS. Bond strength of a novel universal resin cement to dentin with or without an adhesive bonding agent: An in vitro study. *J Contemp Dent Pract*. 2023;24(10):725–728. doi:10.5005/jp-journals-10024-3568
16. Ustun S, Ayaz EA. Effect of different cement systems and aging on the bond strength of chairside CAD–CAM ceramics. *J Prosthet Dent*. 2021;125(2):334–339. doi:10.1016/j.prosdent.2019.11.025
17. Małysa A, Weżgowiec J, Danel D, Boening K, Walczak K, Więckiewicz M. Bond strength of modern self-adhesive resin cements to human dentin and different CAD/CAM ceramics. *Acta Bioeng Biomech*. 2020;22(2):25–34. PMID:32868946.
18. Malysa A, Wezgowiec J, Orzeszek S, Florjanski W, Zietek M, Wieckiewicz M. Effect of different surface treatment methods on bond strength of dental ceramics to dental hard tissues: A systematic review. *Molecules*. 2021;26(5):1223. doi:10.3390/molecules26051223
19. Malysa A, Wezgowiec J, Grzebieluch W, Danel DP, Wieckiewicz M. Effect of thermocycling on the bond strength of self-adhesive resin cements used for luting CAD/CAM ceramics to human dentin. *Int J Mol Sci*. 2022;23(2):745. doi:10.3390/ijms23020745
20. Frankenberger R, Taschner M, Garcia-Godoy F, Petschelt A, Krämer N. Leucite-reinforced glass ceramic inlays and onlays after 12 years. *J Adhes Dent*. 2008;10(5):393–398. PMID:19058686.
21. Frankenberger R, Lohbauer U, Taschner M, Petschelt A, Nikolaenko SA. Adhesive luting revisited: Influence of adhesive, temporary cement, cavity cleaning, and curing mode on internal dentin bond strength. *J Adhes Dent*. 2007;9 Suppl 2:269–273. PMID:18340985.
22. Zhang CY, Cheng YL, Tong XW, Yu H, Cheng H. In vitro cytotoxicity of self-adhesive dual-cured resin cement polymerized beneath three different cusp inclinations of zirconia. *Biomed Res Int*. 2019;2019:7404038. doi:10.1155/2019/7404038
23. Kirsten M, Matta RE, Belli R, et al. Hygroscopic expansion of self-adhesive resin cements and the integrity of all-ceramic crowns. *Dent Mater*. 2018;34(8):1102–1111. doi:10.1016/j.dental.2018.04.008
24. Hitz T, Stawarczyk B, Fischer J, Hämmerle CH, Sailer I. Are self-adhesive resin cements a valid alternative to conventional resin cements? A laboratory study of the long-term bond strength. *Dent Mater*. 2012;28(11):1183–1190. doi:10.1016/j.dental.2012.09.006
25. DIN-Taschenbuch 267. *Zahnheilkunde: Werkstoffe*. 2nd ed. Berlin, Germany: DIN Deutsches Institut für Normung; 2010.
26. Tay FR, Carvalho R, Sano H, Pashley DH. Effect of smear layers on the bonding of a self-etching primer to dentin. *J Adhes Dent*. 2000;2(2):99–116. PMID:11317405.
27. Zhang C, Degrange M. Shear bond strengths of self-adhesive luting resins fixing dentine to different restorative materials. *J Biomater Sci Polym Ed*. 2010;21(5):593–608. doi:10.1163/156856209X431640
28. Sabatini C, Patel M, D'Silva E. In vitro shear bond strength of three self-adhesive resin cements and a resin-modified glass ionomer cement to various prosthodontic substrates. *Oper Dent*. 2013;38(2):186–196. doi:10.2341/11-317-L
29. Phark JH, Duarte S Jr. Microstructural considerations for novel lithium disilicate glass ceramics: A review. *J Esthet Restor Dent*. 2022;34(1):92–103. doi:10.1111/jerd.12864
30. Atalay C, Koc Vural U, Miletic I, Gurgan S. Shear bond strengths of two newly marketed self-adhesive resin cements to different substrates: A light and scanning electron microscopy evaluation. *Microsc Res Tech*. 2022;85(5):1694–1702. doi:10.1002/jemt.24031
31. Kocaağaoğlu H, Manav T, Albayrak H. In vitro comparison of the bond strength between ceramic repair systems and ceramic materials and evaluation of the wettability. *J Prosthodont*. 2017;26(3):238–243. doi:10.1111/jopr.12381
32. Al-Thagafi R, Al-Zordk W, Saker S. Influence of surface conditioning protocols on reparability of CAD/CAM zirconia-reinforced lithium silicate ceramic. *J Adhes Dent*. 2016;18(2):135–141. doi:10.3290/j.jad.a35909
33. Kerschbaum T. *Adhäsivprothetik – Brücken, Attachments, Schienen, Veneers*. München, Germany: Urban & Schwarzenberg; 1995.
34. Wegner SM, Gerdes W, Kern M. Effect of different artificial aging conditions on ceramic–composite bond strength. *Int J Prosthodont*. 2002;15(3):267–272. PMID:12066490.

Original paper

Assessment of the antibacterial activity of glass ionomer cements modified by polyamidoamine and bioactive glass: An in vitro study

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Abstract

Background. Secondary caries is one of the main reasons for the clinical failure in dental restorations. Therefore, it is preferable for restorative materials to possess antibacterial properties, which support a long-lasting restoration.

Objectives. The present in vitro study aimed to evaluate the effect of both the polyamidoamine (PAMAM) liquid and bioactive glass (BAG) powder added to glass ionomer cements (GICs) on their antibacterial properties.

Material and methods. Polyamidoamine was prepared and characterized. Four groups were distinguished, as follows: GI – samples of commercially available GIC (control); GII – samples of GIC mixed with PAMAM; GIII – samples of GIC mixed with BAG; and GIV – samples of GIC mixed with PAMAM and BAG. The biofilm assessment test was conducted using a colony forming unit (CFU) count, and the ion release test was used to quantify the amount of released silica (Si), calcium (Ca), phosphorus (Ph), and sodium (Na) ions in mg/L. Thirty-six samples were prepared for each test. Furthermore, the pH of the soaking solution was measured for each sample in the ion release test. The parametric data was examined using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test.

Results. The biofilm assessment test revealed a significant reduction in the recovered *Streptococcus mutans* counts in all modified groups compared to the control group ($p < 0.05$). Also, the ion release test demonstrated a significant increase in the release of Si and Na ions for all modified groups compared to the control group ($p < 0.05$).

Conclusions. The modification of GICs with PAMAM and BAG enhances their antibacterial properties.

Keywords: bioactive glass, glass ionomer cement, ion release, antibacterial, polyamidoamine

Highlights

- Improving the antibacterial properties of restorative materials may help prevent secondary caries, the most common dental complication.
- The modification of glass ionomer cements (GICs) with polyamidoamine (PAMAM) and bioactive glass (BAG) improves their antibacterial activity and ion release.
- A positive correlation was observed between ion release and antibacterial activity in modified GICs, independent of environmental pH.

Introduction

Glass ionomer cements (GICs) have a wide range of clinical applications due to the possible modification of their physical properties or chemical formulations. Furthermore, the chemical adhesion of GICs to enamel and dentin, the material's good marginal integrity, its dimensional stability at high humidity, as well as its fluoride (F) release and recharge are positive characteristics of the material.[1] Moreover, GICs are considered the best materials of choice for tooth repair by the atraumatic restorative treatment (ART) and the restoration of tooth surfaces with minimal preparations.[2]

Dental caries is one of the most prevalent and significant dental problems. According to the World Health Organization (WHO), approx. 60–90% of children worldwide, and nearly 100% of adults, suffer from dental caries, with secondary caries being one of the main reasons behind the clinical restorative failure of GICs.[3] As *Streptococcus mutans* is among the main microorganisms considered the etiological factors for dental caries,[4] the prevention of bacterial invasion, in the case of microleakage, becomes the key to long-lasting dental restorations.[5] Glass ionomer cements have been reported to be the most cariostatic and antibacterial due to their F release,[6] exhibiting no or low cytotoxicity.[7] However, annual clinical surveys have declared that secondary caries remains the main reason for GIC failure, indicating that the F release from GICs is insufficient to inhibit bacterial growth or prevent the bacterial destruction of the tooth structure.[8]

Glass ionomer restorations with antibacterial properties are highly desirable. Consequently, researchers have investigated various modifications to increase the antibacterial and physical properties of GICs, including the addition of zirconium,[9] glass fiber,[10] hydroxyapatite,[11] bioactive glass particles,[12] casein phosphopeptide-amorphous calcium phosphate,[13] cetrimide, cetylpyridinium chloride, chlorhexidine (CHX),[14] and chitosan (CH).[15]

Bijle et al. investigated the influence of incorporating L-arginine (Arg), a semi-essential amino acid, into GIC.[16] They found that adding 4% Arg to GIC increased its antibacterial activity via a biofilm modulatory effect for microbial homeostasis.[16] Additionally, Neelima et al. investigated the antimicrobial efficacy of conventional GIC with the addition of propolis, CH and CHX against *S. mutans* and *Lactobacillus acidophilus*.[17] It was concluded that GIC with CHX revealed higher antimicrobial activity against both strains. *Streptococcus mutans* and *L. acidophilus* were effectively inhibited by GIC with propolis and GIC with CH.

In several studies, bioactive glass (BAG) has been added to GICs to improve their bioactivity and capacity for tooth regeneration. Additionally, it has demonstrated antimicrobial properties within the oral cavity and low cytotoxicity to dental pulp cells.[18] Polyamidoamine (PAMAM) dendrimer, on the other hand, is a highly developed polymer with reactive endings. Among the PAMAM dendrimers that harbor a variety of functional groups, the amino-terminated PAMAM dendrimers (PAMAM-NH_2) revealed the strongest antibacterial activity. Functional amine groups get adsorbed to the bacterial cell surface, diffusing through the cell wall, with subsequent bacterial death.[19] The antibacterial mechanism of PAMAM dendrimers reveals an apparent lack of inducing bacterial resistance to antibiotics,[20] a phenomenon that may have negative consequences for society.[21] With an increase in the generation number (G) of PAMAM, the number of functional groups doubles.[22] It has been stated that G2 or higher generation of PAMAM-NH_2 can significantly damage bacteria, while G3 or higher generation of PAMAM-NH_2 has the potential to damage mammalian cells.[23] Therefore, it has been proposed that a range of generations of PAMAM-NH_2 exists between both thresholds, possibly having significant antibacterial activity while maintaining tolerable toxicities. According to previous studies, G3 or higher generation of PAMAM-NH_2 dendrimers exhibited excellent antibacterial activities, though they also demonstrated high toxicity.[23]

Gou et al. examined the long-term antimicrobial effect and antiproteolytic potential of PAMAM-NH_2 cavity cleanser, evaluating its binding capacity to dentin surfaces to fulfill these effects.[24] For antibacterial testing, colony forming unit (CFU) counts and live/dead bacterial staining were performed. Antibacterial testing indicated that PAMAM-NH_2 significantly inhibited bacterial growth on the dentin disks compared with the control group, which exhibited a similar level of antibacterial activity to that of 2% CHX ($p > 0.05$). Also, evidence has demonstrated the low cytotoxicity level of PAMAM-NH_2 toward human dental pulp cells.[24]

To the best of our knowledge, no published study has evaluated the effect of adding both PAMAM and BAG on the antibacterial properties of GICs. Therefore, given the importance of antibacterial properties in determining the success and longevity of dental restorations, this study was conducted to evaluate the effect of the addition of both G2 PAMAM-NH_2 liquid and BAG powder to GICs on their antibacterial properties. The effect of pH and ion release ability of the modified GICs on their antibacterial properties was also determined.

Material and methods

According to the calculated sample size, a total of 72 samples were prepared. The materials used in the study are listed in Table 1. First, a pilot study was conducted through a biofilm assessment test (CFU counts) to select the optimum concentration of PAMAM (4, 8, or 16 multiple its minimum bactericidal concentration, which was equal to 6.2%, 12.4% and 24.9% by volume (v), respectively) that would be added to the cement liquid, and the optimum concentration of BAG (5% or 10% by weight (w)) that would be mixed with the cement powder.

Based on the results of the pilot study, the addition of 6.2% PAMAM and 5% BAG was discarded due to their ineffective antibacterial effect, while 12.4% and 24.9% concentrations of PAMAM were selected for incorporation into the cement liquid. Additionally, 10% BAG was added to the cement powder.

Synthesis of polyamidoamine

To obtain the desired second-generation (G2) PAMAM-NH_2 dendrimer, the hyperbranched PAMAM polymer was synthesized by 4 steps of successive Michael addition and amidation reactions. The starting reactants were ethylenediamine as the core and methyl acrylate. Firstly, the Michael addition reaction was executed in a 500-mL round flask, in accordance with the following protocol: 7.5 mL (0.12 mol) of ethylene diamine in 100 mL of methanol was added dropwise to a solution of 42 mL (1.25 eq, 0.46 mol) of methyl acrylate in 25 mL of methanol over 1 h at 0°C. The mixture was stirred for 48 h at room temperature. The reaction mixture was then subjected to a process of evaporation using a rotary evaporator (R-210 Buchi; BÜCHI Labortechnik GmbH, Essen, Germany) to achieve a state of complete dryness. The addition step was followed by the amidation step, which entailed adding a solution of ethylene diamine in 50 mL of methanol at 0°C to 12.5 g (0.03 mol) of the methyl-terminated core in 50 mL of methanol. The reaction mixture was heated to room temperature and stirred for 12 h. The unreacted amine and methanol were removed under a vacuum. Two consequent steps of the Michael addition and amidation reactions were carried out as previously described.[25] The G2 PAMAM-NH_2 was prepared and characterized by Fourier transform infrared analysis (FTIR) (wavelength range: 400–4,000 cm^{-1}, spectrum resolution: 4 cm^{-1}) (VERTEX 80 V; Bruker, Bremen, Germany) and proton nuclear magnetic resonance (^1H NMR) (Jeol 500 MHz; Jeol, Freising, Germany).

Grouping of the study samples

A total of 72 samples were divided into groups and subgroups, based on the volume percentage of PAMAM liquid (L2) added to the GIC liquid (L1), and the BAG powder (P2) added to the GIC powder (P1). The groups were prepared as follows (Fig. 1):

- GI: 100% P1 mixed with with 100% L1;
- GII:
  - GII (A): 100% P1 mixed with L1:L2 at a ratio of 87.52:12.48;
  - GII (B): 100% P1 mixed with L1:L2 at a ratio of 75:25;
- GIII: P1:P2 at a ratio of 90:10 mixed with 100% L1;
- GIV:
  - GIV (A): P1:P2 at a ratio of 90:10 mixed with L1:L2 at a ratio of 87.52:12.48;
  - GIV (B): P1:P2 at a ratio of 90:10 mixed with L1:L2 at a ratio of 75:25.

Table 1. Characteristics of the materials used in the study

Material	Composition	Presentation	Manufacturer	Batch No.
Glass ionomer filling material (GC Gold Label 9)	–	powder/liquid	GC Corporation, Tokyo, Japan	1903071
BAG nanoparticles	46.1% SiO_2, 26.9% CaO, 24.4% Na_2O, 2.6% P_2O_5	powder	Laboratory at the Department of Chemistry, Faculty of Science, Suez Canal University, Ismailia, Egypt	–
Methyl acrylate	99% contain ≤100 ppm of monomethyl ether hydroquinone as an inhibitor	liquid	Sigma-Aldrich, Darmstadt, Germany	M27301
Ethylenediamine	ReagentPlus®, ≥99% synonym: 1,2-diaminoethane	liquid	Sigma-Aldrich, Darmstadt, Germany	E26266
Methane	Messer® CAN Gas, 99.99%	gas	Sigma-Aldrich, Darmstadt, Germany	769126
Ethanol	absolute, for HPLC, ≥99.8% (GC)	liquid	Sigma-Aldrich, Darmstadt, Germany	34870

BAG – bioactive glass (prepared by the sol–gel technique); HPLC – high-performance liquid chromatography; GC – gas chromatography.

Preparation of samples

According to the manufacturer's instructions, the GIC samples were prepared by mixing at a powder–liquid (P/L) ratio of 3.6 to 1.0 (g/g). A digital balance (A200S; Sartorius, Göttingen, Germany) was used to weigh each sample individually. The P/L ratio was consistent across samples in each group. For the samples modified with PAMAM liquid and BAG powder, the GIC liquid was manually mixed with PAMAM, and the GIC powder was manually mixed with the BAG particles. Afterward, the cement powder was mixed with the cement liquid to form a paste, which was placed in a Teflon mold with specific dimensions, according to each test.

Bacterial strain and growth conditions

Streptococcus mutans ATCC 25175 was used as the test organism in the study, and tryptic soy broth (TSB) supplemented with 0.3% w/v yeast extract was employed as its culture medium. Whenever necessary, TSB was supplemented with 2% w/v agar. *Streptococcus mutans* was cultured in a 5% carbon dioxide incubator (Binder GmbH, Ulm, Germany) at 37°C under microaerophilic conditions.

Antibacterial tests

Determination of the minimum inhibitory concentration and minimum bactericidal concentration

The antibacterial activity of PAMAM against *S. mutans* was assessed. According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of PAMAM were determined by the broth microdilution method.[26,27]

GIC biofilm assessment through CFU count

A total of 36 samples (6 disks/group) were prepared according to the manufacturer's instructions in a custom Teflon mold with a diameter of 7.5 mm and a thickness of 1.5 mm. The materials were mixed by a single operator, packed into the mold, covered, and pressed flat with a glass slide.

The test was conducted within the first 24 h after sample preparation, with slight modifications. Briefly, the disks from each group were placed in a 2-mL Eppendorf tube containing 500 µL of TSB supplemented with 0.3% w/v yeast extract and inoculated with a 10% v/v *S. mutans* suspension (10^7 CFU/mL). In each trial, 2 disks of each group were tested. Afterward, the Eppendorf tubes were incubated for 24 h at 37°C in a 5% carbon dioxide incubator (Binder GmbH). Growth control was performed by incubating 2 Eppendorf tubes without adding disks to the bacterial suspension.[28]

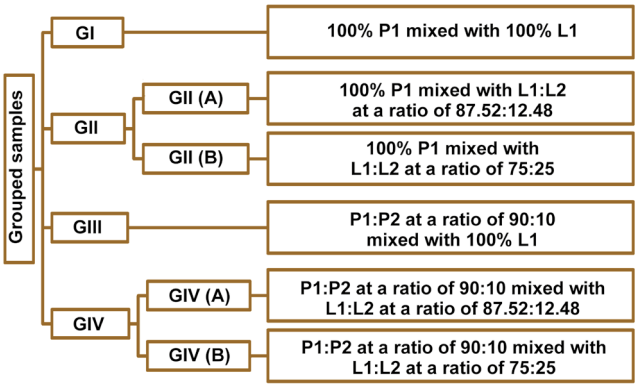

Fig. 1. Grouping of the study samples

G – group; P1 – glass ionomer cement (GIC) powder; P2 – bioactive glass (BAG) powder; L1 – GIC liquid; L2 – polyamidoamine (PAMAM) liquid.

After incubation, each disk was rinsed with 1 mL of sterile phosphate-buffered saline (PBS) and vortexed for 10 min at maximum speed in a new 2-mL Eppendorf tube containing 500 µL of PBS. The resulting bacterial suspensions were subjected to a 10-fold dilution with sterile PBS. Ten microliters from each dilution were spotted on tryptic soy agar plates supplemented with 0.3% w/v yeast extract, for the purpose of the viable count. The CFU of *S. mutans*/disk were counted, and the results were tabulated and statistically analyzed.

Growth control tubes were used to measure the bacterial count and to verify whether the experimental conditions would affect the growth of *S. mutans*.

Ion release test

A total of 36 samples (6 disks/group) were prepared using a split Teflon mold with a diameter of 7.5 mm and a thickness of 1.5 mm. During the preparation of the samples, a convenient length of stainless steel orthodontic ligature wire was inserted into the sample before its final setting. A 10-mm piece of stainless steel wire was projected from the ring to ensure proper handling of the sample and its complete immersion in the deionized water (Fig. 2).

The soaking water was collected after 7 days. The analyzed solution was used to quantify the released silica (Si), calcium (Ca), phosphorus (Ph), and sodium (Na) ions in mg/L using inductively coupled plasma optical emission spectrometry (ICP-OES) (Agilent 5100; Agilent

Fig. 2. Glass jars containing the ion release test samples

Technologies Inc., Petaling Jaya, Malaysia).[29] The results were tabulated and statistically analyzed.

Measurement of the pH

The soaking solution obtained from each sample of the ion release test was further used to measure its pH with an Adwa AD8000 electrode (Adwa Instruments, Nușfalău, Romania) by the immersion in the central part of the solution. Assessments of pH were performed 24 h after specimen preparation.[30] The electrode was cleaned and recalibrated, and the results were tabulated and statistically analyzed.

Statistical analysis

The numerical data was assessed for normality by checking its distribution and using Kolmogorov–Smirnov and Shapiro–Wilk tests. All values were expressed as mean ±standard deviation (*M* ±*SD*). The SPSS Statistics for Windows software, v. 17.0 (SPSS Inc., Chicago, USA) was used for the analysis. The parametric data was examined with one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant. Pearson's correlation and linear regression analysis were used to test the relation between parameters.

Results

Characterization of the prepared PAMAM dendrimers

Fourier transform infrared analysis

The FTIR spectrum for the prepared PAMAM is shown in Fig. 3. The peaks at 3,289 cm^{-1}, 2,944–2,836 cm^{-1} and 1,629 cm^{-1}, were assigned to N–H, C–H Aliphatic and C=O, respectively, indicating the main constituent chemical functional groups of the prepared PAMAM.

Proton nuclear magnetic resonance

The ^{1}H NMR spectrum for the prepared PAMAM liquid is shown in Fig. 4. The peaks at 4.67 δ, 3.15 δ and 2.61 δ, were assigned to NH, CH_2–N and CH_2–CO, respectively, ensuring thorough characterization of the composition of the prepared PAMAM.

Antibacterial tests

Minimum inhibitory concentration and minimum bactericidal concentration

The recorded MIC and MBC for PAMAM against *S. mutans* were 0.78125 ±0% v/v and 1.5625 ±0% v/v, respectively. The MBC was used to determine the optimal concentration of PAMAM that would be added to GICs.

Biofilm assessment assay

The results of the biofilm assessment test are listed in Table 2. A significant decrease in the number of recovered bacteria was detected in all modified groups compared to the control group (GI) ($p < 0.05$). In addition, there was a significant reduction in bacterial counts in GIV (B) in comparison to GII (A) ($p < 0.05$).

The experimental conditions did not affect the growth of *S. mutans*, as indicated by the bacterial count recovered from the growth control tubes (log_{10} 8.33 ±0.124 CFU).

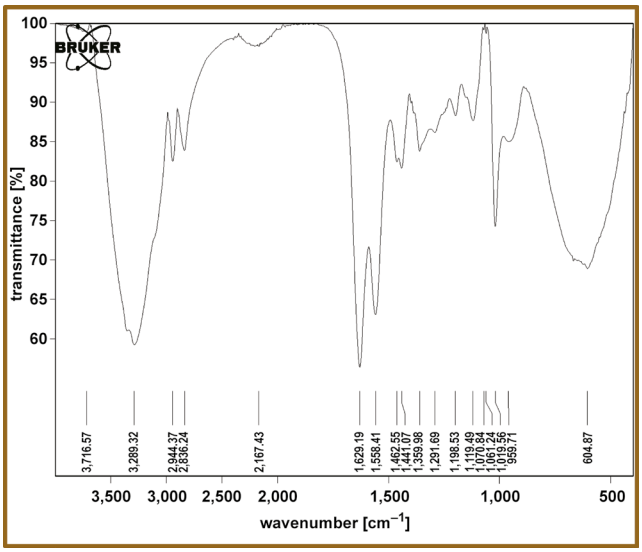

Fig. 3. Fourier transform infrared analysis (FTIR) spectrum for the second generation (G2) amino-terminated polyamidoamine (PAMAM) dendrimer (PAMAM-NH_2) (VERTEX 80 V; Bruker, Bremen, Germany)

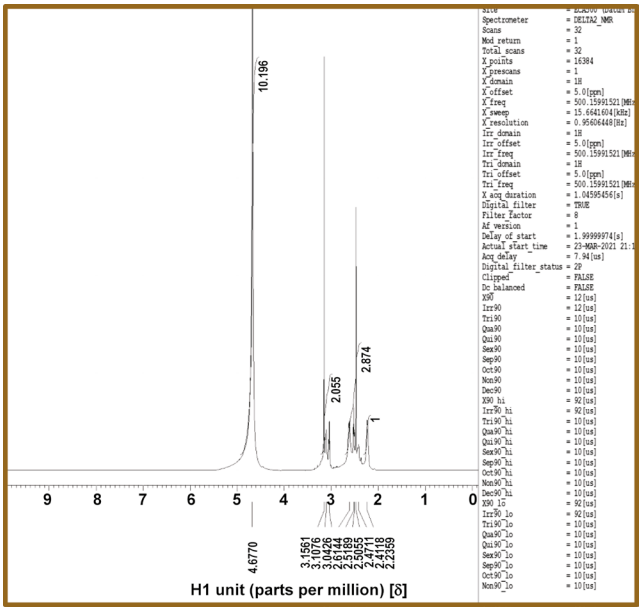

Fig. 4. Proton nuclear magnetic resonance (^{1}H NMR) spectrum for the prepared polyamidoamine (PAMAM) liquid

Table 2. Results of the biofilm assessment test and the ion release test

Group		Biofilm [log_{10} CFU/disk] *M ±SD*	Silica ions [mg/L] *M ±SD*	Sodium ions [mg/L] *M ±SD*	Calcium ions [mg/L] *M ±SD*	Phosphorus ions [mg/L] *M ±SD*
GI		6.15 ±0.31[bcdef]	9.38 ±3.23[bcdef]	16.83 ±2.01[bcdef]	0.31 ±0.15[def]	0.61 ±0.37[cdef]
GII	GII (A)	4.47 ±1.23[af]	35.14 ±4.86[aef]	30.73 ±5.11[ac]	0.55 ±0.34[df]	1.67 ±0.32[def]
	GII (B)	3.62 ±0.38[a]	45.23 ±1.68[ade]	48.92 ±4.13[abdef]	0.56 ±0.22[df]	2.49 ±0.48[aef]
GIII		3.93 ±0.38[a]	27.85 ±6.68[acef]	36.62 ±2.44[ace]	5.94 ±2.06[abc]	3.68 ±1.03[abh]
GIV	GIV (A)	3.88 ±0.66[a]	61.01 ±8.07[abcd]	25.34 ±2.35[acdf]	5.6 ±1.92[af]	5.13 ±1.37[abc]
	GIV (B)	2.99 ±1.11[ab]	50.85 ±8.46[abd]	32.41 ±4.69[ace]	6.76 ±1.03[abce]	4.16 ±0.76[abc]

M – mean; *SD* – standard deviation; CFU – colony forming units; G – group. Different uppercase letters indicate statistically significant differences between the groups ($p < 0.05$). Letters [a], [b], [c], [d], [e], and [f] indicate significant differences when compared to GI, GII (A), GII (B), GIII, GIV (A), and GIV (B), respectively.

Ion release test

The results of the ion release test are listed in Table 2.

Silica and sodium ion release

There was a significant increase in the release of Si and Na ions in the solution for all modified groups compared to the control group (GI) ($p < 0.05$). A significant increase in the amount of Si ions released in GIV (A) was observed in comparison to GI, GII (A and B) and GIII ($p < 0.05$). At the same time, GII (B) showed a significant increase in the amount of released Na ions compared to the other groups ($p < 0.05$).

Calcium ion release

There was a significant increase in the amount of the Ca ions released in GIII and GIV (A and B) compared to the control group (GI) ($p < 0.05$). An insignificant difference in the amount of Ca ions was observed between GI and GII (A and B) ($p > 0.05$).

Phosphorus ion release

There was a significant increase in the amount of the released Ph ions in GII (B), GIII and GIV (A and B), compared to the control group (GI) ($p < 0.05$). An insignificant difference in the amount of released Ph ions was noted between GI and GII (A) ($p > 0.05$).

pH test of the soaking solution after 24 h

The pH test results for the soaking solution after 24 h in the various investigated groups are listed in Table 3. There was an insignificant difference in the pH between the control group and all modified groups ($p > 0.05$). A significant decrease in the pH of the solution of GIV (A and B) was observed when compared to the GII (A) and GIII solutions ($p < 0.05$).

Discussion

The most common reason for restorative failure is secondary caries, which is mainly caused by oral bacteria.[31] In recent years, a significant body of research has been conducted to develop antibacterial dental restorative materials that could enable the eradication of the cause of dental caries.[32] An effective antibacterial lining material may address potential issues such as the remnant cariogenic microorganisms after partial caries removal.[33] Therefore, this study aimed to investigate the interaction of *S. mutans* with GICs modified with BAG and PAMAM in terms of bacterial growth.

The conventional glass ionomer (GC Gold Label 9, high-strength posterior restorative) was selected due to its high P/L ratio, which offers significant advantages over other commonly used restorative dental materials. It has become the material of choice in the ART technique.[34,35] Furthermore, it is a resin-free material presented in powder and liquid formats for manual mixing, and it sets chemically, without the need for light curing. This characteristic facilitates the ART procedures for young and old individuals, as well as in low-income countries.[36] The material was preferred over encapsulated materials because it more readily permitted modification of its constituents by the inclusion of PAMAM and BAG additives.

In this study, G2 PAMAM-NH_2 was selected as an antibacterial agent due to its 16 primary amine surface functional groups, which result in lower toxicity,[23] and its relatively simple and cheap synthesis when compared to higher generations.

In the present study, finishing and polishing procedures for the samples were not performed to avoid surface contamination before interaction with the *S. mutans*

Table 3. Results of the pH test

Group		pH of the soaking solution *M ±SD*
GI		6.17 ±0.05
GII	GII (A)	6.18 ±0.09[ef]
	GII (B)	6.15 ±0.05
GIII		6.23 ±0.11[ef]
GIV	GIV (A)	5.85 ±0.12[bd]
	GIV (B)	5.83 ±0.19[bd]

Different uppercase letters indicate statistically significant differences between the groups ($p < 0.05$). Letters [b], [d], [e], and [f] indicate significant differences when compared to GII (A), GIII, GIV (A), and GIV (B), respectively.

biofilm, which should require sterilization of the samples.[37] Sterilization methods were not preferred as they might have an impact on the properties of the studied restorative materials, degradation, crack formation, as well as other surface modifications of the GIC.[38,39]

In the pilot study, MBC was selected as the starting concentration of PAMAM on 4 occasions due to its established efficacy in in vivo settings.[40] As no effective difference in the recovered bacterial count was achieved between the control group and the modified groups, the added concentrations of PAMAM and BAG were doubled.

In the biofilm assessment assay, the control group exhibited antibacterial properties, likely attributable to the release of F in the vicinity of the cement, and the low pH of GICs during the setting process. This result is consistent with the findings reported by Shashibhushan et al.[41] and Krämer et al.[42]

Also, the antibacterial properties of GII might be due to the antibacterial properties of PAMAM-NH_2 and its ability to disrupt the bacterial cell,[20] an outcome that aligns with the observations reported by Maleki et al.[43] and Matboo et al.[44] The results of the ion release test indicated a greater release of Si, Na, Ca, and Ph ions from GII compared to the control group, a phenomenon that could potentially explain their enhanced antibacterial properties.

Additionally, the antibacterial properties of GII may be due to the antibacterial effects of BAG particles on *S. mutans*.[45] The ion release test results indicate a higher Si, Na, Ca, and Ph ion release, which may account for its enhanced antibacterial effect compared to the control group. Furthermore, it was revealed that needle-like BAG debris on the bacterial surfaces disrupted the bacterial structure, indicating that debris from BAG-modified GIC was among the causes of bacterial death.[46]

Moreover, the elevated antibacterial properties of GIV, which surpass those of all other groups, might result from the increased release of Si, Ca and Ph ions, as well as the potential antibacterial effect of the added BAG and PAMAM.

In the ion release test, the modified groups showed higher Si, Na, Ca, and Ph ion release in comparison to the control group. This may be due to the addition of PAMAM aid in the initial attack of the glass particles by both polyacrylic acid and PAMAM, causing the chelation of more ions that ionize during the test. It has been stated that PAMAM with different terminal groups possesses calcium coordination sites that attract free calcium and phosphate from the saliva.[47] Furthermore, the bioactive nature of BAG is related to its ion release ability.[48] The modification of GIC by adding BAG particles composed of Si, Na, Ca, and Ph,[36] which dissolve in the solution, may lead to an enhanced amount of ion release compared to GIC alone. The results on Si ion release were consistent with those reported by Yli-Urpo et al.,[29] though they did not align with their observations on Ca and Ph ion release.

The pH of the soaking solution was measured to investigate its effect on the antibacterial activity of the modified GIC. The study revealed an insignificant difference in the pH of the soaking solution after 24 h between the control group and all modified groups. This finding suggests that the antibacterial activity of the modified groups may be due to their chemical composition and ion-releasing ability, rather than the pH.

Limitations

The present research is not without its limitations. First, sterilization of the samples was not performed before conducting the antibacterial test. Also, biocompatibility tests for the PAMAM and BAG-modified GIC samples were not conducted. In addition, the pilot study indicated that the added percentages of PAMAM and BAG had to be selected as the least effective antibacterial concentration to prevent alterations in mechanical properties and setting time.

Conclusions

The modification of GICs with PAMAM and BAG has been demonstrated to enhance their antibacterial properties. Also, it has been shown that the higher the ion release ability of the modified GICs, the more pronounced their antibacterial effect, independent of pH. The hypothesis of the present study was rejected, as the PAMAM and BAG added to GICs enhanced their antibacterial properties.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Kovarik RE, Haubenreich JE, Gore D. Glass ionomer cements: A review of composition, chemistry, and biocompatibility as a dental and medical implant material. *J Long Term Eff Med Implants*. 2005;15(6):655–671. doi:10.1615/JLongTermEffMedImplants.v15.i6.80
2. Vermeersch G, Leloup G, Delmée M, Vreven J. Antibacterial activity of glass-ionomer cements, compomers and resin composites: Relationship between acidity and material setting phase. *J Oral Rehabil*. 2005;32(5):368–374. doi:10.1111/j.1365-2842.2004.01300.x
3. Kale S, Kakodkar P, Shetiya S, Abdulkader R. Prevalence of dental caries among children aged 5–15 years from 9 countries in the Eastern Mediterranean Region: A meta-analysis. *East Mediterr Heal J*. 2020;26(6):726–735. doi:10.6719/emhj.20.050
4. Krzyściak W, Jurczak A, Kościelniak D, Bystrowska B, Skalniak A. The virulence of *Streptococcus mutans* and the ability to form biofilms. *Eur J Clin Microbiol Infect Dis*. 2014;33(4):499–515. doi:10.1007/s10096-013-1993-7
5. Murata H, Koepsel RR, Matyjaszewski K, Russell AJ. Permanent, non-leaching antibacterial surfaces—2: How high density cationic surfaces kill bacterial cells. *Biomaterials*. 2007;28(32):4870–4879. doi:10.1016/j.biomaterials.2007.06.012
6. Svenson S, Tomlia DA. Dendrimers in biomedical applications—reflections on the field. *Adv Drug Deliv Rev*. 2005;57(15):2106–2129. doi:10.1016/j.addr.2005.09.018
7. Skośkiewicz-Malinowska K, Mysior M, Rusak A, Kuropka P, Kozakiewicz M, Jurczyszyn K. Application of texture and fractal dimension analysis to evaluate subgingival cement surfaces in terms of biocompatibility. *Materials (Basel)*. 2021;14(19):5857. doi:10.3390/ma14195857
8. Weng Y, Guo X, Zhao J, Gregory RL, Xie D. A PQAS-containing glass-ionomer cement for improved antibacterial function. *J Biomed Sci Eng*. 2010;3(10):955–962. doi:10.4236/jbise.2010.310126
9. Gu YW, Yap AUJ, Cheang P, Khor KA. Zirconia–glass ionomer cement–a potential substitute for Miracle Mix. *Scr Mater*. 2005;52(2):113–116. doi:10.1016/j.scriptamat.2004.09.019
10. Hammouda IM. Reinforcement of conventional glass ionomer restorative material with short glass fibers. *J Mech Behav Biomed Mater*. 2009;2(1):73–81. doi:10.1016/j.jmbbm.2008.04.002
11. Lucas ME, Arita K, Nishino M. Toughness, bonding and fluoride-release properties of hydroxyapatite-added glass ionomer cement. *Biomaterials*. 2003;24(21):3787–3794. doi:10.1016/s0142-9612(03)00260-6
12. Yli-Urpo H, Lassila LVJ, Närhi T, Vallittu PK. Compressive strength and surface characterization of glass ionomer cements modified by particles of bioactive glass. *Dent Mater*. 2005;21(3):201–209. doi:10.1016/j.dental.2004.03.006
13. Al Zraikat H, Palamara JEA, Messer HH, Burrow MF, Reynolds EC. The incorporation of casein phosphopeptide-amorphous calcium phosphate into a glass ionomer cement. *Dent Mater*. 2011;27(3):235–243. doi:10.1016/j.dental.2010.10.008
14. Tüzüner T, Ulusu T. Effect of antibacterial agents on the surface hardness of a conventional glass-ionomer cement. *J Appl Oral Sci*. 2012;20(1):45–49. doi:10.1590/s1678-77572012000100009
15. Paradowska-Stolarz A, Wieckiewicz M, Owczarek A, Wezgowiec J. Natural polymers for the maintenance of oral health: Review of recent advances and perspectives. *Int J Mol Sci*. 2021;22(19):10337. doi:10.3390/ijms221910337
16. Bijle MN, Ekambaram M, Lo ECM, Yiu CKY. Antibacterial and mechanical properties of arginine-containing glass ionomer cements. *Dent Mater*. 2020;36(9):1226–1240. doi:10.1016/j.dental.2020.05.012
17. Neelima B, Reddy JS, Singh PT, Suhasini K, Hemachandrika I, Hasanuddin S. Comparative evaluation of antimicrobial efficacy of glass ionomer cement added with propolis, chitosan, and chlorhexidine against *Streptococcus mutans* and *Lactobacillus acidophilus*: An in vitro study. *J Indian Soc Pedod Prev Dent*. 2020;38(4):367–373. doi:10.4103/JISPPD.JISPPD_322_20
18. Salehi S, Davis HB, Ferracane JL, Mitchell JC. Sol-gel-derived bioactive glasses demonstrate antimicrobial effects on common oral bacteria. *Am J Dent*. 2015;28(2):111–115. PMID:26087578.
19. Chen CZ, Cooper SL. Interactions between dendrimer biocides and bacterial membranes. *Biomaterials*. 2002;23(16):3359–3368. doi:10.1016/s0142-9612(02)00036-4
20. Xue X, Chen X, Mao X, et al. Amino-terminated generation 2 poly(amidoamine) dendrimer as a potential broad-spectrum, nonresistance-inducing antibacterial agent. *AAPS J*. 2013;15(1):132–142. doi:10.1208/s12248-012-9416-8
21. Kohanski MA, DePristo MA, Collins JJ. Sublethal antibiotic treatment leads to multidrug resistance via radical-induced mutagenesis. *Mol Cell*. 2010;37(3):311–320. doi:10.1016/j.molcel.2010.01.003
22. Sadekar S, Ghandehari H. Transepithelial transport and toxicity of PAMAM dendrimers: Implications for oral drug delivery. *Adv Drug Deliv Rev*. 2011;64(6):571–588. doi:10.1016/j.addr.2011.09.01
23. Lopez AI, Reins RY, McDermott AM, Trautner BW, Cai C. Antibacterial activity and cytotoxicity of PEGylated poly(amidoamine) dendrimers. *Mol Biosyst*. 2009;5(10):1148–1156. doi:10.1039/b904746h
24. Gou Y, Jin W, He Y, et al. Effect of cavity cleanser with long-term antibacterial and anti-proteolytic activities on resin–dentin bond stability. *Front Cell Infect Microbiol*. 2021;11:784153. doi:10.3389/fcimb.2021.784153
25. Tomalia DA, Baker H, Dewald J, et al. Dendritic macromolecules: Synthesis of starburst dendrimers. *Macromolecules*. 1986;19(9):2466–2468. doi:10.1021/ma00163a029
26. Albash R, Abdellatif MM, Hassan M, Badawi NM. Tailoring terpesomes and leciplex for the effective ocular conveyance of moxifloxacin hydrochloride (comparative assessment): In-vitro, ex-vivo, and in-vivo evaluation. *Int J Nanomedicine*. 2021;16:5247–5263. doi:10.2147/IJN.S316326
27. Humphries RM, Ambler J, Mitchell SL, et al.; CLSI Methods Development and Standardization Working Group of the Subcommittee on Antimicrobial Susceptibility Testing. CLSI Methods development and standardization working group best practices for evaluation of antimicrobial susceptibility tests. *J Clin Microbiol*. 2018;56(4):e01934–17. doi:10.1128/JCM.01934-17
28. Noori AJ, Kareem FA. The effect of magnesium oxide nanoparticles on the antibacterial and antibiofilm properties of glass-ionomer cement. *Heliyon*. 2019;5(10):e02568. doi:10.1016/j.heliyon.2019.e02568
29. Yli-Urpo H, Vallittu PK, Närhi TO, Forsback AP, Väkiparta M. Release of silica, calcium, phosphorus, and fluoride from glass ionomer cement containing bioactive glass. *J Biomater Appl*. 2004;19(1):5–20. doi:10.1177/0885328204044538
30. Lehmann A, Nijakowski K, Nowakowska M, Woś P, Misiaszek M, Surdacka A. Influence of selected restorative materials on the environmental pH: In vitro comparative study. *Appl Sci*. 2021;11(24):11975. doi:10.3390/app112411975
31. Sakaguchi RL. Review of the current status and challenges for dental posterior restorative composites: Clinical, chemistry, and physical behavior considerations. Summary of discussion from the Portland Composites Symposium (POCOS) June 17–19, 2004, Oregon Health and Science University, Portland, Oregon. *Dent Mater*. 2005;21(1):3–6. doi:10.1016/j.dental.2004.10.008
32. Imazato S. Antibacterial properties of resin composites and dentin bonding systems. *Dent Mater*. 2003;19(6):449–457. doi:10.1016/s0109-5641(02)00102-1
33. Prabhakar AR, Prahlad D, Kumar SR. Antibacterial activity, fluoride release, and physical properties of an antibiotic-modified glass ionomer cement. *Pediatr Dent*. 2013;35(5):411–415. PMID:24290552.
34. Arita K, Yamamoto A, Shinonaga Y, et al. Hydroxyapatite particle characteristics influence the enhancement of the mechanical and chemical properties of conventional restorative glass ionomer cement. *Dent Mater J*. 2011;30(5):672–683. doi:10.4012/dmj.2011-029
35. De Caluwé T, Vercruysse CWJ, Fraeyman S, Verbeeck RMH. The influence of particle size and fluorine content of aluminosilicate glass on the glass ionomer cement properties. *Dent Mater*. 2014;30(9):1029–1038. doi:10.1016/j.dental.2014.06.003
36. Prentice LH, Tyas MJ, Burrow MF. The effect of particle size distribution on an experimental glass-ionomer cement. *Dent Mater*. 2005;21(6):505–510. doi:10.1016/j.dental.2004.07.016
37. Carlén A, Nikdel K, Wennerberg A, Holmberg K, Olsson J. Surface characteristics and in vitro biofilm formation on glass ionomer and composite resin. *Biomaterials*. 2001;22(5):481–487. doi:10.1016/s0142-9612(00)00204-0
38. Farrugia C, Cassar G, Valdramidis V, Camilleri J. Effect of sterilization techniques prior to antimicrobial testing on physical properties of dental restorative materials. *J Dent*. 2015;43(6):703–714. doi:10.1016/j.jdent.2015.03.012

39. Wezgowiec J, Paradowska-Stolarz A, Malysa A, Orzeszek S, Seweryn P, Wieckiewicz M. Effects of various disinfection methods on the material properties of silicone dental impressions of different types and viscosities. *Int J Mol Sci*. 2022;23(18):10859. doi:10.3390/ijms231810859
40. Salem MA, El-Shiekh RA, Hashem RA, Hassan M. In vivo antibacterial activity of star anise (*Illicium verum* Hook.) extract using murine MRSA skin infection model in relation to its metabolite profile. *Infect Drug Resist*. 2021;14:33–48. doi:10.2147/IDR.S285940
41. Shashibhushan KK, Basappa N, Reddy VVS. Comparison of antibacterial activity of three fluorides and zinc releasing commercial glass ionomer cements on strains of mutans streptococci: An in vitro study. *J Indian Soc Pedod Prev Dent*. 2008;26 Suppl 2:S56–S61. PMID:19075449.
42. Krämer N, Schmidt M, Lücker S, Domann E, Frankenberger R. Glass ionomer cement inhibits secondary caries in an in vitro biofilm model. *Clin Oral Investig*. 2018;22(2):1019–1031. doi:10.1007/s00784-017-2184-1
43. Maleki A, Ahmadi Jebelli M, Hayati B, Daraei H, Gharibi F. Microbial deactivation properties of generation 2 and 4 poly(amidoamine) dendrimers on common bacteria found in the aqueous environment. *Antimicrob Resist Infect Control*. 2015;4(Suppl 1):P35. doi:10.1186/2047-2994-4-S1-P35
44. Matboo SA, Nazari S, Mohammadi L, et al. In vitro anti-bacterial effect of nano-polyamidoamine-G5 dendrimer. *Koomesh*. 2019;21(1):171–180. https://brieflands.com/articles/koomesh-153054 . Accessed April 3, 2023.
45. Yli-Urpo H, Närhi T, Söderling E. Antimicrobial effects of glass ionomer cements containing bioactive glass (S53P4) on oral micro-organisms in vitro. *Acta Odontol Scand*. 2003;61(4):241–246. doi:10.1080/00016350310004719
46. Hu S, Chang J, Liu M, Ning C. Study on antibacterial effect of 45S5 Bioglass. *J Mater Sci Mater Med*. 2009;20(1):281–286. doi:10.1007/s10856-008-3564-5
47. Tao S, Fan M, Xu HHK, et al. The remineralization effectiveness of PAMAM dendrimer with different terminal groups on demineralized dentin in vitro. *RSC Adv*. 2017;7:54947–54955. doi:10.1039/C7RA11844A
48. Alamri A, Salloot Z, Alshaia A, Ibrahim MS. The effect of bioactive glass-enhanced orthodontic bonding resins on prevention of demineralization: A systematic review. *Molecules*. 2020;25(11):2495. doi:10.3390/molecules25112495

Original paper

Novel demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs) for enhanced bone repair

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Abstract

Background. The number of patients with bone defects is increasing. The treatment of damaged bones or bone defects is essential. Bone graft materials are frequently used in bone repair procedures. Researchers are attempting to replace damaged or defective bones with artificial ones, while also striving to improve the mechanical and biological compatibility of the scaffolds.

Objectives. The study aimed to establish and validate novel demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs) for enhancing bone repair.

Material and methods. Demineralized freeze-dried bovine bone xenograft nanoparticles were extracted from bovine femoral bone. The physicochemical and biochemical properties of both native and demineralized freeze-dried materials were evaluated using Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), field-emission scanning electron microscopy (FESEM), and the Brunauer–Emmett–Teller (BET) analysis. Enzyme-linked immunosorbent assay (ELISA) kits were utilized to determine the content of type 1 collagen (Col-1) and bone morphogenetic protein-2 (BMP-2), water content percentage (WCP), and enzymatic degradation.

Results. The physicochemical analysis revealed non-porous DFDBBX-NPs with near spherical shapes of various sizes. The dried sample presented the nanoparticles agglomerated together, with an average size of 10–50 nm. The nanoparticles exhibit a type IV isotherm with an H3 hysteresis loop. They have a BET-specific surface area of 3 m^2/g and a pore diameter of approx. 5.9 nm. The bioactive content of BMP-2 was higher than that of Col-1 in the DFDBBX-NPs. The DFDBBX-NP scaffold exhibited a slow rate of enzymatic degradation (0.098–0.240% over 14 days) and high water absorption (WCP ~202–215%).

Conclusions. Demineralized freeze-dried bovine bone xenograft nanoparticles demonstrated remarkable potential for the development of new bone grafts.

Keywords: bone graft, demineralized bovine bone, osteogenic, bone regeneration

Highlights

- Demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs) exhibit favorable physicochemical properties and nanoscale dimensions, supporting their suitability as bone graft materials.
- The osteoinductive potential of DFDBBX-NPs is supported by the presence of bioactive proteins, with BMP-2 levels exceeding those of collagen-1.
- DFDBBX-NP scaffolds demonstrate controlled disintegration and effective water absorption, indicating their potential for bone tissue regeneration and restoration.

Introduction

Bone grafting is one of the most commonly performed tissue transplantation procedures. Bone graft materials are widely used to repair bone defects resulting from various pathological conditions.[1,2] In dental practice, bone damage can be attributed to numerous factors, including tumors, cysts, periodontitis, or trauma. Therefore, there is an increasing demand for research on the development of bone graft materials. An ideal bone graft material must be osteoinductive, biocompatible, osteoconductive, bioresorbable, porous, easy to use, mechanically resistant, cost-effective, and have a bone-like structure. Several types of bone grafts are available, namely autografts, allografts, xenografts, and alloplasts.[3–5] Autografts are considered the gold standard in bone grafting because they contain viable bone cells that contribute to osteogenesis and release growth factors that promote osteoinduction.[6,7] Although autogenous grafts are currently employed in dentistry, their usage requires a second surgery. The development of bone graft substitutes is therefore necessary to reduce the complexity of bone extraction for autograft.[8,9] These issues and the shortcomings of allogeneic transplantation, such as the transmission of possible infections and the lack of osseointegration with host tissues, have prompted researchers and clinicians to explore novel graft solutions using challenging biomaterial techniques.[10]

Biomaterials are being continuously developed as an alternative option to restore damaged bone tissue. Xenograft was designed to overcome the disadvantages of autografting, regarded as the gold standard. Xenografts have similar structure to human bone and are abundant in resources. A demineralized freeze-dried bovine bone xenograft (DFDBBX) facilitates osteoconduction, thereby promoting the regeneration of alveolar bone. Recent studies have reported that deproteinized bovine bone mineral (DBBM) has poor biodegradation properties.[11,12] Researchers started focusing on the regeneration of bone tissue in a potential 3D scaffold, a field of study known as bone tissue engineering (BTE).[13–15] Demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs) have been developed as alternative bovine-derived bone substitutes. These nanoparticles are produced through the demineralization of bovine bone, which removes the inorganic mineral phase while preserving organic components that contain osteoinductive growth factors, such as bone morphogenetic proteins (BMPs). The ideal scaffold should exhibit optimal compatibility, be non-toxic to cells and biodegradable. For this reason, a physical and chemical characterization of DFDBBX-NPs is necessary.

Material and methods

Synthesis of DFDBBX-NPs

The study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia (approval No. 176/EC/KEPK/07/2022). The demineralized freeze-dried bovine bone was derived from bovine femoral bone after removal of all adherent soft tissues. The bone was cut into fragments measuring 3 mm × 3 mm, and washed using hydrogen peroxide solution to ensure complete removal of blood and any residual fatty tissue. Subsequently, the samples were rinsed in a solution of sodium chloride (NaCl) and aquadest to remove residual peroxide, and soaked in 1% hydrogen chloride (HCl) solution for 24 h, until the minerals disappeared. The samples were freeze-dried using a lyophilizer system (FreeZone® 4.5; Labconco Corporation, Kansas City, USA).

Characterization of DFDBBX-NPs

Fourier-transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) of the samples was conducted using a spectrometer (Thermo Scientific™ Nicolet™ iS10; Thermo Fisher Scientific, Waltham, USA) at a scanning range of 400–4,000 cm^{-1} and with a resolution of 4 cm^{-1}.

X-ray diffraction

X-ray diffraction (XRD) measurements were carried out using powder diffraction (X'pert^3 Powder; Malvern Panalytical Ltd, Malvern, UK). The obtained XRD pattern was compared to the International Centre for Diffraction Data (ICDD) patterns. The analysis was performed on an XRD machine (D8 ADVANCE; Bruker, Billerica, USA).

Surface morphology

Field-emission scanning electron microscopy (FESEM) images of the samples were obtained using a FlexSEM 1000 microscope (Hitachi Ltd., Tokyo, Japan). A Leo 1450VP microscope (ZEISS, Oberkochen, Germany) was used to further evaluate the morphology and size of the nanoparticles. Samples were mounted directly onto aluminum stubs, to which a gold coating was applied prior to examination. Micrographs were recorded at magnifications of ×10,000, ×20,000 and ×50,000.

Survey area and pore size analysis

The Brunauer–Emmett–Teller (BET) surface area of the samples was measured using a BELSORP-mini II (BEL Japan, Inc., Toyonaka, Japan) based on nitrogen gas adsorption–desorption isotherms at 77 K and evaluated using the BET equation. Demineralized freeze-dried bovine bone xenograft nanoparticles were inserted into a sample holder, heated and degassed using a sample cleaning station to clean the samples. Next, liquid nitrogen was poured into a dewar for the subsequent analysis with a gas pycnometer (AccuPyc III; Micromeritics®, Norcross, USA). The BET-specific surface area, adsorption–desorption isotherms, pore diameter, and pore volume were recorded. The Barrett–Joyner–Halenda (BJH) desorption method was used to determine the pore diameter and volume of the samples.

ELISA testing

Enzyme-linked immunosorbent assay (ELISA) kits (type 1 collagen (Col-1): E1378; BMP-2: E2240) were purchased from Bioassay Technology Laboratory (Shanghai, China). The kits were used to quantify the content of anti-Col-1 and BMP-2 in demineralized and control samples. Briefly, approx. 50 mg of DFDBBX-NPs was transferred into a 50 µL of the extraction buffer. Subsequently, 10 µL of anti-Col-1 antibody and 50 µL of streptavidin-horseradish peroxidase (HRP) were added to each of the samples. After incubation at 37°C for 60 min, the plate was unsealed and washed 5 times using wash buffer, followed by 3 freeze–thaw cycles. Then, the samples were sonicated using an ultrasonic tip to extract proteins. The total protein content was determined by means of a microplate reader set (Thermo Scientific Multiskan GO; Thermo Fisher Scientific) at 450 nm within 10 min after the addition of the stop solution.

Water content percentage

The scaffold used as a sample was weighed to determine its initial weight (W_0). Then, it was placed into an Eppendorf tube and immersed in 1 mL of phosphate buffer saline (PBS) solution (pH = 7.4) for 3, 7 and 14 days at 37°C. Next, the scaffold was slowly extracted using small tweezers to avoid any potential damage, dried with filter paper, and weighed to determine its final weight (W_t). The weight loss was calculated using the following formula (Equation 1):

$$\text{weight loss [\%]} = (W_0 - W_t)/W_0 \times 100 \quad (1)$$

Enzymatic degradation

The second scaffold used as a sample was weighed to determine its initial weight (W_0). The scaffold was immersed in 1 mL of solution (PBS and 50 µg/mL collagenase enzyme) for 3, 7 and 14 days. Then, the scaffold was removed from the medium, washed with distilled water, frozen at −80°C, and freeze-dried for 24 h (2 cycles). The final weight (W_t) was recorded. The procedure was repeated for 3 days and 7 days. The degradation rate was calculated using the following formula (Equation 2):

$$\text{degradation rate [\%]} = (W_0 - W_t)/W_0 \times 100 \quad (2)$$

Statistical analysis

The collected data was entered into a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, USA) and analyzed using the IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA). Descriptive data was presented as frequency and percentages (*n* (%)) for categorical variables and as median values with interquartile range (*Me* (*IQR*)) for continuous variables. The Kruskall–Wallis test was used to compare the variables between the study groups. A *p*-value <0.05 was considered statistically significant.

Results

FTIR analysis

The FTIR of DFDBBX-NPs exhibited the most intense bands at 400–4,000 cm^{-1}, with transmittance values of approx. 70–100%. As shown in Fig. 1, the DFDBBX-NPs exhibit prominent absorption peaks at 3,539.38, 3,485.37, 3,375.43, 3,319.49, 3,302.13, 3,223.05, 3,203.76, 3,165.19, 3,084.18, 3,068.75, 2,960.73, 2,879.72, 2,318.44, 2,268.29, 2,131.34, 2,090.84, 1,990.54, 1,415.75, 1,336.67, 1,234.44, 1,205.51, 1,141.86, 1,035.05, 991.41, 960.55, 873.75, 752.24, 601.79, and 569.00 cm^{-1}. Hydroxyapatite (HA) absorption bands were detected in the ranges of 500–700 cm^{-1} and 900–1,200 cm^{-1}, while collagen-associated bands appeared primarily between 1,200–1,700 cm^{-1} and

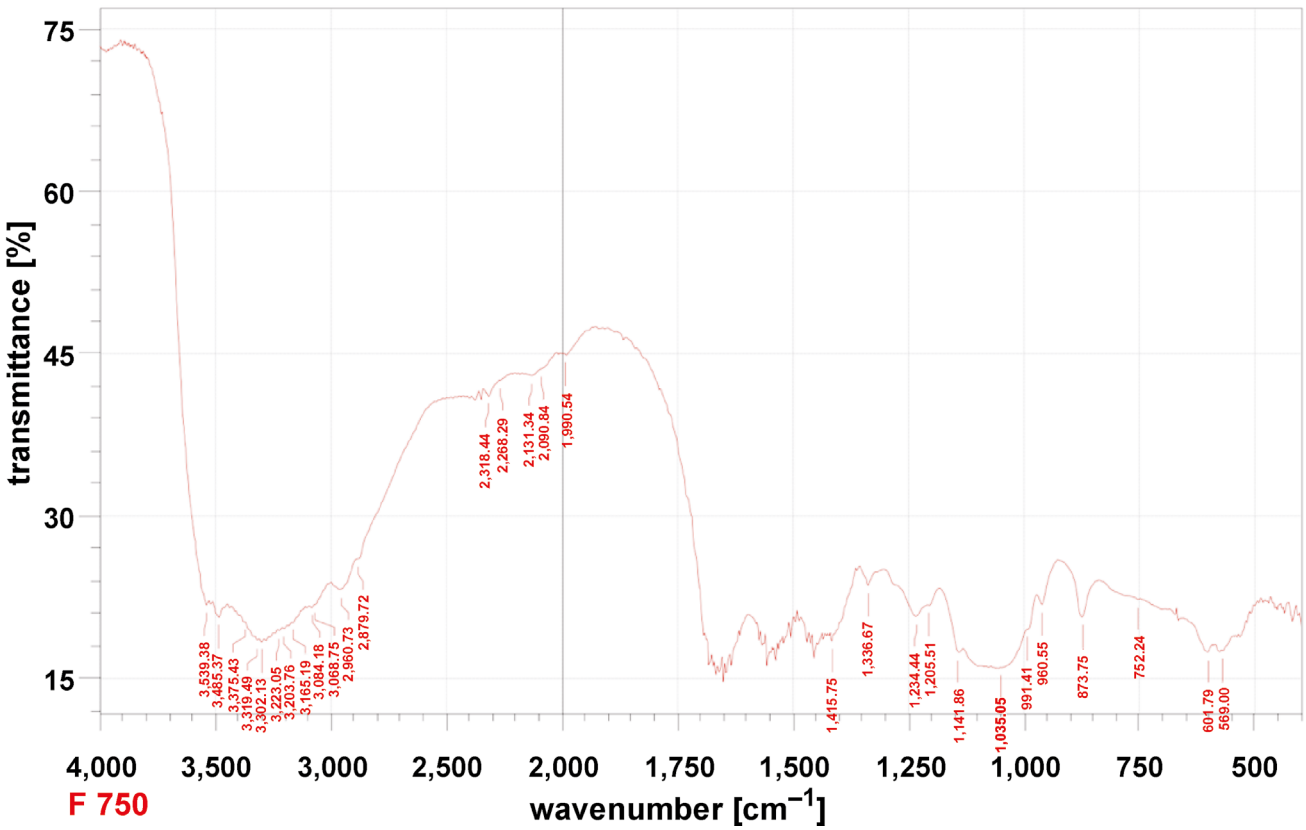

Fig. 1. Fourier transform infrared spectroscopy (FTIR) analysis

2,800–3,700 cm^{-1}. Carbonate apatite was identified by characteristic bands at 870–880 cm^{-1} (single band) and 1,400–1,450 cm^{-1} (double band). The results of FTIR indicate that the absorption peaks at 1,205.51, 1,234.44, 1,336.67, and 1,415.75 cm^{-1} were attributed to amide III and CH_2 bending vibrations of collagen, while the broad bands found at 3,302.13 and 3,319.49 cm^{-1} were associated with amide A (N–H stretching). Additional peaks in the range of 2,879.72–3,539.38 cm^{-1} were related to amide A and B vibrations, confirming the presence of Col-1 in the DFDBBX-NPs. These findings indicate that the demineralization process effectively preserved the collagen structure within the scaffold, while the freeze-drying process retained proteins and minerals.

Table 1. X-ray diffraction (XRD) peak positions of demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs)

Position [°2Th.]	Height [cts]	FWHM left [°2Th.]	d-spacing [Å]	Relative intensity [%]
11.6921	21.98	0.3149	7.56893	25.41
21.0432	34.27	0.2362	4.22186	39.61
25.9501	27.58	0.1968	3.43360	31.88
29.3894	38.38	0.1968	3.03916	44.37
30.6090	23.56	0.2362	2.92078	27.23
31.8002	86.50	0.3936	2.81404	100.00
34.1693	30.88	0.3936	2.62416	35.70
37.1137	5.99	0.4723	2.42246	6.93
39.6543	11.73	0.6298	2.27292	13.56
46.7130	13.99	0.4723	1.94460	16.17
49.4208	8.70	0.4723	1.84421	10.05
53.2930	8.68	0.4723	1.71898	10.04

FWHM – full width at half maximum.

X-ray diffraction

The results of the XRD analysis of DFDBBX-NPs present the chemical composition of the samples (Table 1). Pure HA and brushite were the primary crystalline phases, with HA being the most abundant (69%), followed by brushite (31%). The crystalline phases revealed strong peak characteristics at 2θ values of 11.69°, 21.04°, 25.95°, 29.38°, 30.60°, 31.80°, 34.17°, 37.11°, 39.65°, 46.71°, 49.42°, and 53.29°, with a purity value of 100%.

Surface morphology

As shown in Fig. 2 and Fig. 3, the resulting particles exhibit non-uniform and irregular morphologies, with evident agglomeration. The formation of agglomerates is attributed to the wide particle size distribution. The aggregation is likely driven by direct interparticle interactions, such as Van der Waals forces or chemical bonds. Figure 4 presents nanosized particles at a magnification of ×50,000. Nanoparticles are generally defined as solid

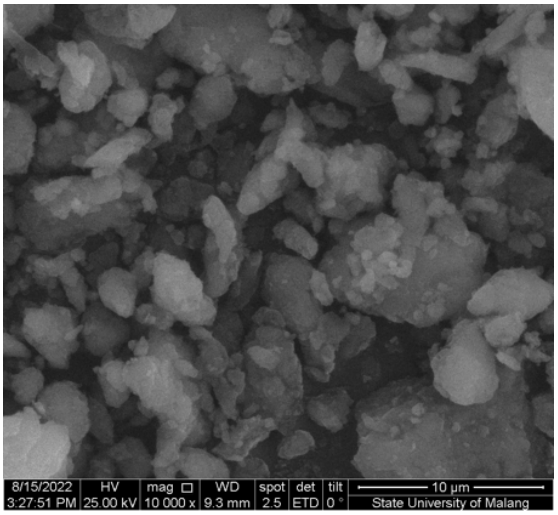

Fig. 2. Field-emission scanning electron microscopy (FESEM) micrograph showing the surface morphology of demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs) (×10,000 magnification)

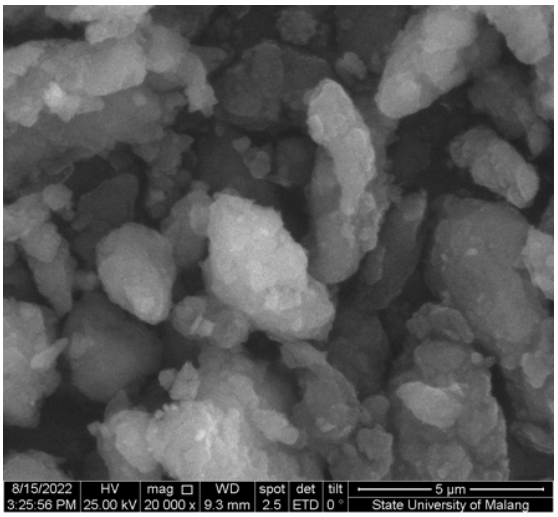

Fig. 3. Field-emission scanning electron microscopy (FESEM) micrograph showing the surface morphology of demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs) (×20,000 magnification)

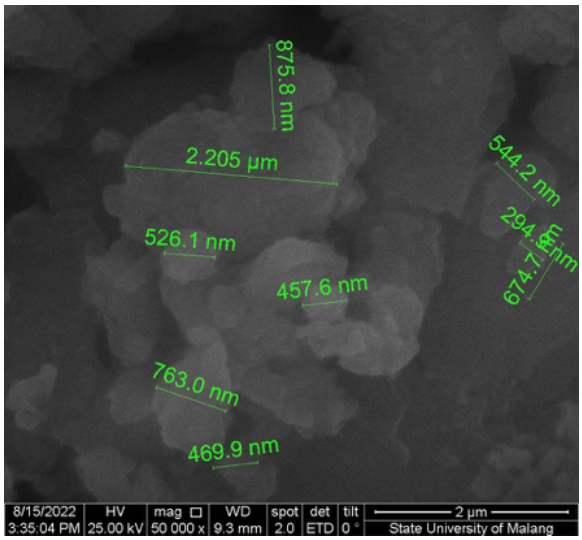

Fig. 4. Field-emission scanning electron microscopy (FESEM) micrograph showing the surface morphology and particle size of demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs) (×50,000 magnification)

particles with sizes ranging from 10 nm to 1,000 nm. However, pronounced aggregation at this magnification indicates the coexistence of both nano- and microscale particles, making precise size determination difficult and suggesting that only a fraction of the particles fall within the microscale range.

Survey area analysis

The BET surface area analyses of the sample were conducted, and its BET adsorption and desorption isotherms with pore size distribution are given in Fig. 5 and Fig. 6. The type IV isotherm with an H_3 hysteresis loop, based on the International Union of Pure and Applied Chemistry

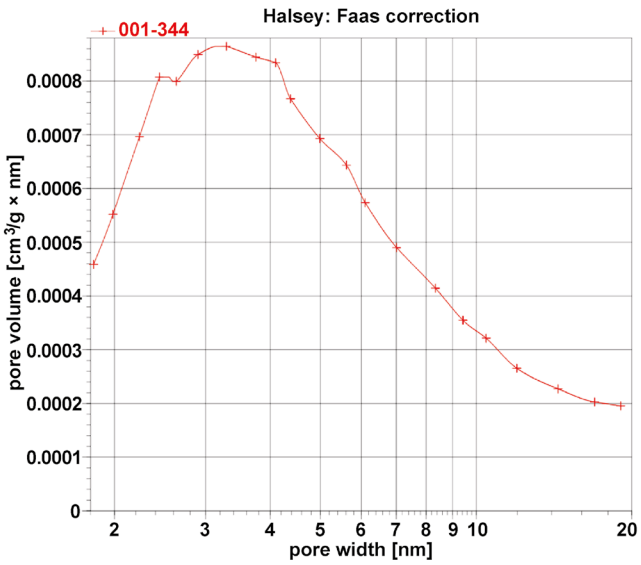

Fig. 5. Barrett–Joyner–Halenda (BJH) adsorption isotherm and pore size distribution of demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs)

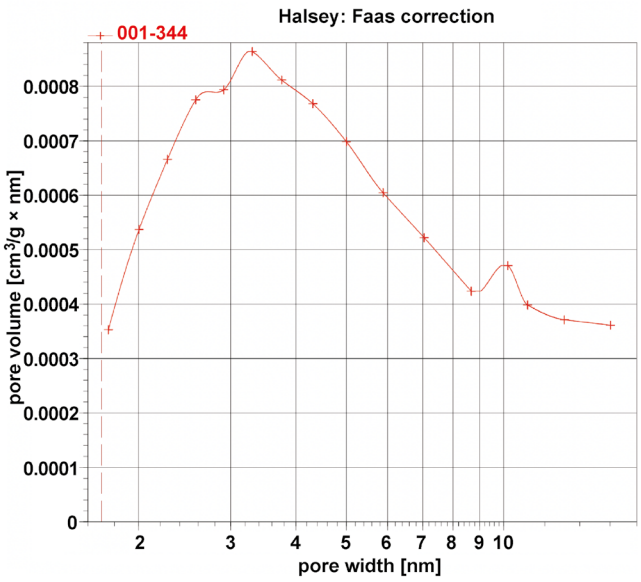

Fig. 6. Barrett–Joyner–Halenda (BJH) desorption isotherm and pore size distribution of demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs)

(IUPAC) classification, is indicative of mesoporous materials, which consist of aggregates of plate-like particles, resulting in slit-shaped pores. The BET-specific surface area of the sample is 3 m^2/g with a BJH pore diameter of 5.9 nm, and a total pore volume of 0.008680 cm^3/g.

Water content percentage

The water content percentage (WCP) in the DFDBBX-NP scaffold was evaluated to determine its capacity for water absorption. The average WCP values are presented in Table 2. Scaffolds with higher water content generally exhibit improved cell infiltration and nutrient transport. The average WCP of the DFDBBX-NP scaffold on the 3rd day was 201.98%; on the 7th day, it was 201.19%; and on the 14th day, it was 214.50% (Fig. 7).

Table 2. Water content percentage (WCP) in the demineralized freeze-dried bovine bone xenograft nanoparticle (DFDBBX-NP) scaffold over the study period

Time	W_0 [mg]	W_t [mg]	WCP [%]
Day 3	12.65	38.20	201.98
Day 7	15.25	45.95	201.19
Day 14	13.10	41.20	214.50

W_0 – initial weight; W_t – final weight.

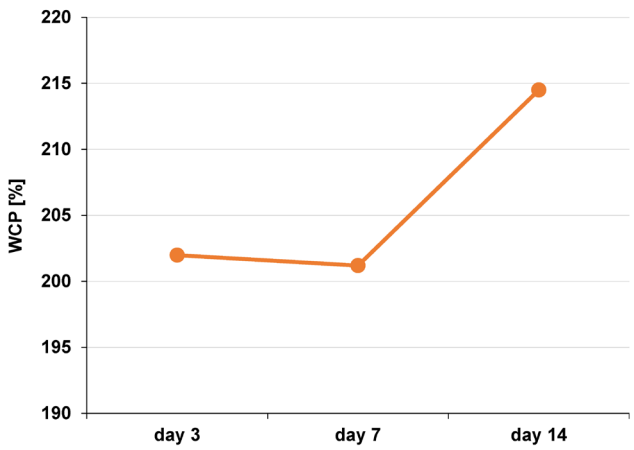

Fig. 7. Water content percentage (WCP) in the demineralized freeze-dried bovine bone xenograft nanoparticle (DFDBBX-NP) scaffold over the study period

Enzymatic degradation

The scaffold was monitored to determine its enzymatic degradation rate. The DFDBBX-NP scaffold exhibited degradation rates of 0.098% after 3 days, 0.207% after 7 days and 0.240% after 14 days of immersion (Fig. 8). The mean values are presented in Table 3.

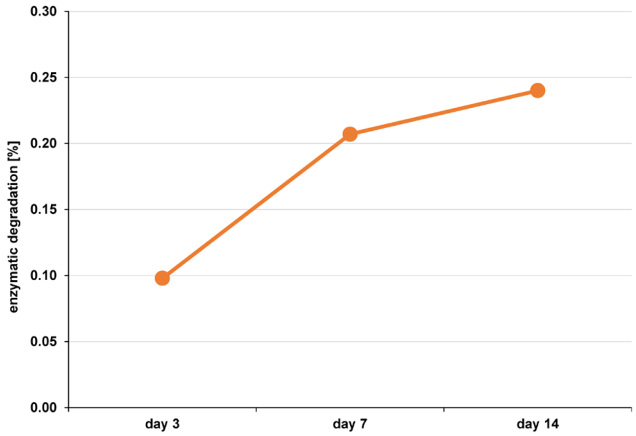

Fig. 8. Enzymatic degradation of the demineralized freeze-dried bovine bone xenograft nanoparticle (DFDBBX-NP) scaffold over the study period

Table 3. Enzymatic degradation of the demineralized freeze-dried bovine bone xenograft nanoparticle (DFDBBX-NP) scaffold after 3, 7 and 14 days of incubation

Time	W_0 [mg]	W_t [mg]	Enzymatic degradation [%]
Day 3	19.20	17.55	0.098
Day 7	19.10	16.40	0.207
Day 14	27.75	23.20	0.240

ELISA testing

The BMP-2 content (0.52 ng/mg) in the DFDBBX-NPs was higher than that of Col-1 (0.31 ng/mg) (Fig. 9). Demineralized bovine bone xenografts are organic materials with inherent osteogenic potential. They contain bioactive molecules such as Col-1 and BMP-2, which play essential roles in osteoinduction and osteoconduction.

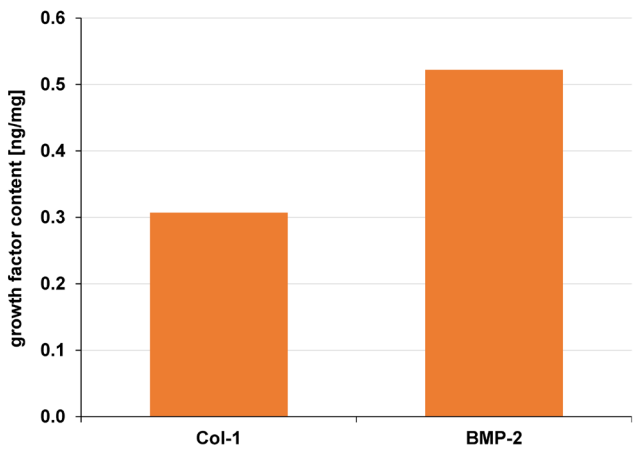

Fig. 9. Growth factor content in the demineralized freeze-dried bovine bone xenograft nanoparticles (DFDBBX-NPs) as determined by enzyme-linked immunosorbent assay (ELISA)

Col-1 – type 1 collagen; BMP-2 – bone morphogenetic protein-2.

Discussion

The FTIR analysis of the DFDBBX-NPs demonstrated characteristic peaks corresponding to both organic and inorganic components. Absorption bands at 2,593 cm^{-1} and 2,002 cm^{-1} indicates interactions between the organic matrix and minerals. The peak at 1,791 cm^{-1} is attributed to the absorption of the C=O group from the primary amide, whereas the peak of 1,620 cm^{-1} indicates the deformation of NH_2 from amide I and C–N stretching vibrations of the secondary amide. Asymmetric stretching vibrations observed at 1,147 cm^{-1} and 1,089 cm^{-1} are associated with the absorption from the PO_4 group. The presence of the PO_4 group is supported by stretching and bending vibrations with absorption bands at 966 cm^{-1} and 574 cm^{-1}. Bending vibrations of the CO_3 group were identified at 1,456 cm^{-1} and 873 cm^{-1}. Overall, the FTIR confirms the presence of a mineral matrix primarily composed of phosphate and calcium ions forming HA crystals ($Ca_{10}(PO_4)_5(OH)_2$), bicarbonate, and an organic phase consisting of the C=O group, CN, C–O–C, and OH.[16]

The XRD characterization graph of the DFBBX-NPs revealed the presence of HA and brushite. Hydroxyapatite, known in the literature as hydroxylapatite, is a rare natural mineral with the chemical formula of $Ca_5(PO_4)_3(OH)$, which is commonly expressed as $Ca_{10}(PO_4)_6(OH)_2$ to indicate that the crystalline unit cell consists of 2 entities. Due to its chemical similarity to the mineral component of human bone and teeth, HA is widely used, especially as a filling material, to repair bone defects.[17] The high crystallinity of HA contributes to its enhanced mechanical strength. However, if the crystallinity is too high, it will be more difficult to absorb it into the body than bone apatite. The crystallinity level of bovine HA can be controlled by adjusting the annealing temperature and holding time during the preparation of HA from bovine.[18,19]

The nanoparticles used as delivery carriers must possess controlled morphology, uniform size distribution and high biocompatibility. Agglomeration occurs as a result of clumping during cooling or storage. Such aggregation is very difficult to avoid due to attractive forces between particles that cause it.[20,21] However, sonication can effectively reduce agglomeration by separating particle clusters through cavitation-induced shear forces.

In order to minimize the aggregation of nanoparticles, the use of an appropriate capping agent is required. Capping agents regulate nanoparticle growth, aggregation and physicochemical characteristics. Various capping agents are employed in the synthesis of nanoparticles, including surfactants, small ligands, polymers, dendrimers, cyclodextrins, and polysaccharides. Common examples include polyethylene glycol (PEG), polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), bovine serum albumin (BSA), ethylenediaminetetraacetic acid (EDTA), chitosan, and plant extracts. Additionally, capping agents enhance the biomedical performance of nanoparticles by reducing toxicity and increasing biocompatibility and bioavailability in living cells.[22–24]

The water content percentage reflects the scaffold's ability to absorb and retain water. In the present study, the swelling ratio graph results with WCP demonstrated comparable trends. The hydrophilicity of the scaffold is essential for interaction with biological fluids and facilitates cell migration into the scaffold. The highest WCP value was observed at 14 days (214.50%). The treatment group exhibited significantly higher hydrophilicity than the control group at 3, 7 and 14 days. Although a slight increase in WCP was observed in the control group between days 3 and 7, the difference was not significant. In the 2nd week, the capacity for water absorption was no longer increasing. Beyond serving as a medium for cell infiltration, the water absorption capacity of the scaffold supports the distribution of nutrients, metabolites and growth factors through the extracellular media. Therefore, hydrophilic properties are essential for successful bone regeneration. Hydrophobic surfaces tend to promote higher protein adsorption. Protein adsorption on the scaffold surfaces facilitates cell attachment by mediating cell signaling through adhesion receptors, particularly integrins. However, excessive hydrophobicity can lead to dense protein deposition on the scaffold surface.[25]

The enzymatic biodegradation analysis revealed differences in the behavior or pattern of degradation. Over the 14-day observation period, both scaffolds retained more than half of their initial dimensions. An appropriate biodegradation rate is vital to eliminate the need for second surgery. Bone regeneration typically requires at least 6 months, and resorbable scaffolds are expected to function as temporary barriers for approx. 3–4 weeks. Biodegradation is an essential parameter in the occurrence of regeneration. Therefore, synchronization between scaffold degradation and new bone formation is essential to ensure complete resorption upon tissue regeneration. Hydrophilic properties, chemical composition, degree of crystallization, and scaffold geometry influence the degradation rate.[26,27] The FTIR results confirmed collagen as the main component of the scaffold. The incorporation of nanostructured components has been shown to slow degradation by neutralizing acidic degradation byproducts.[27] Furthermore, the modulation of chemical composition, surface morphology, and the incorporation of cross-linking agents can be used to tailor the degradation rate.[28,29]

The presence of growth factors in DFDBBX-NPs was confirmed in this study. Previous reports indicate that the osteogenic potential of DFDBBX is associated with their protein composition, particularly Col-1 and BMPs.[30,31] In the present study, ELISA analysis demonstrated that the BMP-2 content in the DFDBBX-NPs was higher than that of Col-1. These findings suggest that DFDBBX-NPs possess osteogenic potential. Osteoinduction is primarily driven by BMP activity within the organic bone matrix.

The growth factors play a significant role in the osteogenesis of DFDBBX-NPs.[30,31]

Conclusions

Results of the present study demonstrated that DFDBBX-NPs are capable of delivering growth factors at physiologically relevant concentrations, supporting their potential application in bone regeneration.

Ethics approval and consent to participate

The study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia (approval No. 176/EC/KEPK/07/2022).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Suprianto K, Hidayati H, Nilam C, Khairiyah N, Amelia R, Rahmadita S. Hidroksiapatit dari cangkang telur sebagai bone graft yang potensial dalam terapi periodontal. *Majalah Kedokteran Gigi Klinik*. 2019;5(3):76–87. https://journal.ugm.ac.id/mkgk/article/view/65729/31229. Accessed January 1, 2023.
2. Walmsley GG, McArdle A, Tevlin R, et al. Nanotechnology in bone tissue engineering. *Nanomedicine*. 2015;11(5):1253–1263. doi:10.1016/j.nano.2015.02.013
3. Cho YD, Seol YJ, Lee YM, Rhyu IC, Ryoo HM, Ku Y. An overview of biomaterials in periodontology and implant dentistry. *Adv Mater Sci Eng*. 2017;2017(1):1948241. doi:10.1155/2017/1948241
4. Amini Z, Lari R. A systematic review of decellularized allograft and xenograft-derived scaffolds in bone tissue regeneration. *Tissue Cell*. 2021;69:101494. doi:10.1016/j.tice.2021.101494
5. Al-Nawas B, Schiegnitz E. Augmentation procedures using bone substitute materials or autogenous bone – a systematic review and meta-analysis. *Eur J Oral Implantol*. 2014;7 Suppl 2:S219–S234. PMID:24977257.
6. Wang W, Yeung KWK. Bone grafts and biomaterials substitutes for bone defect repair: A review. *Bioact Mater*. 2017;2(4):224–247. doi:10.1016/j.bioactmat.2017.05.007
7. Wychowanski P, Wolinski J, Morawiec T, et al. Preliminary clinical data and the comparison of the safety and efcacy of autogenous bone grafts versus xenograft implantations in vertical bone deficiencies before dental implant installation. *Transplant Proc*. 2020;52(7):2248–2251. doi:10.1016/j.transproceed.2020.02.099
8. Schmidt AH. Autologous bone graft: Is it still the gold standard? *Injury*. 2021;52 (Suppl 2):S18–S22. doi:10.1016/j.injury.2021.01.043
9. Pratiwi AR, Hussein MZ, Istikharoh F, et al. Physicochemical characterization of demineralized freeze dried dentine matrix nanoparticle as new candidate bone graft. *Sys Rev Pharm*. 2023;14(3):159–163. doi:10.31858/0975-8453.14.3.159-163
10. Kouhestani F, Rezai Rad M, Mohaghegh S, Motamedian SR. Effect of metformin on the behavior of dental pulp stem cells cultured on freeze-dried bone allografts. *Dent Med Probl*. 2021;58(3):343–349. doi:10.17219/dmp/131988
11. Baharuddin NA, Kamin S, Samsuddin AR. The use of demineralized freeze-dried bovine bone xenograft in reducing post-surgical periodontal recession. *Annal Dent Univ Malaya*. 2005;12;37–40. https://ejournal.um.edu.my/index.php/adum/article/view/13771/8602. Accessed March 1, 2023.
12. Kamadjaja DB, Harijadi A, Soesilawati P, et al. Demineralized freeze-dried bovine cortical bone: Its potential for guided bone regeneration membrane. *Int J Dent*. 2017;2017:5149675. doi:10.1155/2017/5149675
13. Policarpo Tonelli FM, Policarpo Tonelli FC, Carvalho Ferreira DR, et al. Biocompatibility and functionalization of nanomaterials. In: Ahmad N, Gopinath P, eds. *Intelligent Nanomaterials for Drug Delivery Applications*. Amsterdam, the Netherlands: Elsevier; 2020:85–103.
14. Lichte P, Pape HC, Pufe T, Kobbe P, Fischer H. Scaffolds for bone healing: Concepts, materials and evidence. 2011;42(6):569–573. doi:10.1016/j.injury.2011.03.033
15. Guo Z, Yang C, Zhou Z, Chen S, Li F. Characterization of biodegradable poly(lactic acid) porous scaffolds prepared using selective enzymatic degradation for tissue engineering. *RSC Adv*. 2017;7(54):34063–34070. doi:10.1039/c7ra03574h
16. Bapat RA, Joshi CP, Bapat P, et al. The use of nanoparticles as biomaterials in dentistry. *Drug Discov Today*. 2019;24(1):85–98. doi:10.1016/j.drudis.2018.08.012
17. Santo VE, Gomes ME, Mano JF, Reis RL. From nano- to macro-scale: Nanotechnology approaches for spatially controlled delivery of bioactive factors for bone and cartilage engineering. *Nanomedicine (Lond)*. 2012;7(7):1045–1066. doi:10.2217/nnm.12.78
18. Park HJ, Min KD, Lee MC, et al. Fabrication of 3D porous SF/β-TCP hybrid scaffolds for bone tissue reconstruction. *J Biomed Mater Res A*. 2016;104(7):1779–1787. doi:10.1002/jbm.a.35711
19. Tandon P, Förster G, Neubert R, Wartewig S. Phase transitions in oleic acid as studied by X-ray diffraction and FT-Raman spectroscopy. *J Mol Struc*. 2000;524(1–3):201–215. doi:10.1016/S0022-2860(00)00378-1
20. Figueiredo M, Henriques J, Martins G, Guerra F, Judas F, Figueiredo H. Physicochemical characterization of biomaterials commonly used in dentistry as bone substitutes – comparison with human bone. *J Biomed Mater Res B Appl Biomater*. 2010;92B(2):409–419. doi:10.1002/jbm.b.31529
21. Galia CR, Lourenço AL, Rosito R, Souza Macedo CA, Camargo LMAQ. Physicochemical characterization of lyophilized bovine bone grafts. *Rev Bras Ortop*. 2015;46(4):444–451. doi:10.1016/S2255-4971(15)30260-3
22. Müller-Goymann CC. Physicochemical characterization of colloidal drug delivery systems such as reverse micelles, vesicles, liquid crystals and nanoparticles for topical administration. *Eur J Pharm Biopharm*. 2004;58(2):343–356. doi:10.1016/j.ejpb.2004.03.028
23. Santander-Ortega MJ, Bastos-González D, Ortega-Vinuesa JL, Alonso MJ. Insulin-loaded PLGA nanoparticles for oral administration: An in vitro physico-chemical characterization. *J Biomed Nanotechnol*. 2009;5(1):45–53. doi:10.1166/jbn.2009.022
24. Lin PC, Lin S, Wang PC, Sridhar R. Techniques for physicochemical characterization of nanomaterials. *Biotechnol Adv*. 2014;32(4):711–726. doi:10.1016/j.biotechadv.2013.11.006
25. O'Brien FJ. Biomaterials & scaffolds for tissue engineering. *Mater Today*. 2011;14(3):88–95. doi:10.1016/S1369-7021(11)70058-X

26. Rodríguez-Vázquez M, Vega-Ruiz B, Ramos-Zúñiga R, Saldaña-Koppel DA, Quiñones-Olvera LF. Chitosan and its potential use as a scaffold for tissue engineering in regenerative medicine. *Biomed Res Int*. 2015;2015:821279. doi:10.1155/2015/821279
27. Yassin MAAY. *Surface Hydrophilicity: A Key Factor in Developing Bone Tissue Engineering Constructs* [dissertation]. Bergen, Norway: University of Bergen; 2017.
28. Yassin MA, Leknes KN, Pedersen TO, et al. Cell seeding density is a critical determinant for copolymer scaffolds-induced bone regeneration. *J Biomed Mater Res*. 2015;103(11):3649–3658. doi:10.1002/jbm.a.35505
29. Wu S, Liu X, Yeung KWK, Liu C, Yang X. Biomimetic porous scaffolds for bone tissue engineering. *Mater Sci Eng R Rep*. 2014;80:1–36. doi:10.1016/j.mser.2014.04.001
30. Istikharoh F, Wardani SC, Pratiwi AR, Sujuti H, Mustamsir E. Demineralized freeze-dried bovine bone xenograft enhance osteoblast viability and proliferation for jaw regeneration materials. *J Dentomaxillofac Sci*. 2023;8(2):105–109. doi:10.15562/jdmfs.v8i2.1390
31. Mohonta SK, Maria KH, Rahman S, Das H, Manjura S. Synthesis of hydroxyapatite nanoparticle and role of its size in hydroxyapatite/chitosan–gelatin biocomposite fore bone grafting. *Int Nano Lett*. 2021;11:381–393. doi:10.1007/s40089-021-00347-9

Original paper

Meta-analysis of the global prevalence of oral and facial pain

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Abstract

Background. Pain is one of the most fundamental yet complex human biological experiences. A distinct category within this domain is orofacial pain. The orofacial region is anatomically complex, comprising a dense network of muscles, nerves and blood vessels. This complexity often makes the diagnosis and management of conditions affecting this area particularly challenging for clinicians.

Objectives. The primary aim of this study was to determine the global prevalence of oral and facial pain in the general population and to assess its distribution across different continents.

Material and methods. Literature searches were conducted in the MEDLINE and Scopus databases. This analysis synthesized data from 155 observational studies published between 2004 and 2024, encompassing 1,712,161 participants and 163,154 reported cases. Statistical analyses were performed using the R statistical language.

Results. The pooled global prevalence of concurrent oral and facial pain was 19.19%. By continent, the prevalence rates were as follows: Africa – 27.44%; Asia – 30.78%; Australia – 15.58%; Europe – 12.27%; North America – 14.53%; and South America – 25.61%.

Conclusions. Globally, the prevalence of concurrent oral and facial pain is 19%, compared with 12% for facial pain alone, and 21% for oral pain. Concurrent oral and facial pain was most common among individuals under 18 years of age (24%), decreasing to 16% among those aged 18–60 years and 10% in those aged >60 years. Females reported a higher prevalence (21%) of oral and facial pain than males (15%). Overall, the burden of concurrent oral and facial pain is greatest in Asia and South America, particularly among females, where prevalence exceeds 28%, while the lowest rates are found in Europe, especially among males (8%). The results of the present study should be interpreted with caution due to the high heterogeneity of the included studies. Further epidemiological research is recommended, employing rigorous methodology and adhering to current pain classification systems.

Keywords: prevalence, epidemiology, sex, orofacial pain, facial

Highlights

- Globally, approx. 19% of individuals experience concurrent oral and facial pain, while 21% report oral pain and 12% report facial pain alone.
- The highest prevalence rates are observed in Africa (31% – oral pain; 27% – concurrent oral and facial pain) and Asia (31% – concurrent oral and facial pain), whereas Europe reports the lowest prevalence (12% – concurrent oral and facial pain).
- Oral and facial pain is most prevalent among individuals aged ≤18 years (24%), declining to 16% in adults aged 18–60 years and to 10% in those aged >60 years.
- Females are more frequently affected (21%) than males (15%).
- Longer assessment periods (≥6 months) are associated with higher reported prevalence of concurrent oral and facial pain (>25%) compared with shorter recall periods (1–3 months).

Introduction

Pain is one of the most fundamental yet complex human biological experiences.[1,2] The International Association for the Study of Pain (IASP) defines it as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage".[3] Pain is not merely a reaction to nociceptive stimuli but a multidimensional process integrating biological, psychological and social components.[1–4]

In the context of the present work, it is essential to clearly distinguish between 3 related but distinct terms: "oral pain"; "facial pain"; and "orofacial pain". Oral pain refers specifically to pain experienced within the structures of the oral cavity (e.g., teeth, gingiva, mucosa, tongue), while facial pain describes discomfort perceived in the facial region outside the mouth, such as the cheeks, jaw or periorbital area.[5,6] Orofacial pain, as used in the present study, is an overarching term that encompasses both oral and facial pain, irrespective of whether its origin is dental, musculoskeletal, neuropathic, or idiopathic.[7,8]

Orofacial pain represents a distinct clinical category due to the anatomical complexity of the orofacial region, which contains a dense network of muscles, nerves and blood vessels. This complexity often makes the diagnosis and treatment of conditions affecting this area challenging for clinicians.[7,8] The IASP defines orofacial pain as a frequent form of pain perceived in the face and/or oral cavity.[9] While facial pain of oral origin is commonly associated with intraoral dental or periodontal pathologies, differential diagnostic challenges arise when pain originates from other sources, such as temporomandibular disorders (TMD) or neuropathic pain.[10] Therefore, the distinction between oral, facial and orofacial pain is not only terminological but also clinically significant, as it affects diagnostic strategies and therapeutic approaches.

Patients experiencing orofacial pain often present with impaired mental health, elevated anxiety levels, depression, and perceived stress, all of which significantly impact quality of life.[11,12] Beyond its high prevalence, the clinical significance of orofacial pain lies in its potential to cause long-term disability, reduced masticatory function, impaired communication, and psychosocial burden. Chronic orofacial pain contributes to sleep disturbances, reduced work productivity and increased healthcare utilization, making it a relevant public health issue.[13,14]

Ethnic and cultural variations in pain perception constitute a significant area of investigation in pain medicine and health psychology.[15,16] Multiple studies have demonstrated that ethnic groups may differ in both pain threshold and pain tolerance, stemming from a combination of biological, psychological and sociocultural factors. Genetic predispositions may influence the functioning of nociceptive and pain-modulating systems, whereas cultural norms and behavioral patterns shape how individuals express or suppress pain-related complaints.[1,17–19] In addition to ethnic and cultural determinants, sex has been demonstrated to play a role in orofacial pain perception; estrogen levels have been shown to be associated with pain modulation in the temporomandibular joint and the orofacial region as a whole.[20,21]

Recent evidence indicates substantial variation in the prevalence of orofacial pain across populations and regions. A systematic review conducted by Porporatti et al. reported that, in adults, the prevalence of pain in the oral and facial regions ranges from approx. 1% in burning mouth syndrome to over 80% in orofacial pain associated with cancer treatment. In pediatric populations, the prevalence varies from below 1% for temporomandibular joint osteoarthritis up to 83% for all types of headaches.[22] Another study reported that 19% of individuals with dementia experience orofacial pain.[23] Two meta-analyses published in 2024 identified regional differences in the prevalence of TMD and bruxism.[24,25] The prevalence of TMD was significantly higher in South America (47%) compared to Asia (33%), Europe (29%) and North America (26%).[24] Additionally, the prevalence of bruxism (sleep and awake) was reported as 29% in North America, 25% in South America, 22% in Europe, and 19% in Asia.[25] Further differences were observed in a meta-regression analysis examining the co-occurrence of bruxism and TMD.[26]

However, despite numerous studies addressing individual conditions such as TMD or bruxism, comprehensive population-based data on the global prevalence of orofacial pain as a unified entity remains limited. Previous meta-analyses have focused on specific disorders rather than the overall pain experience, leaving uncertainty regarding how orofacial pain differs across continents and demographic groups.

The present study aims to address this gap by providing the first meta-analytic estimate of the global and continental prevalence of orofacial pain in the general population. A secondary objective is to assess differences in prevalence according to age and sex. The working hypothesis of this study is that the global prevalence of orofacial pain does not exceed 30%. Clarifying these patterns is clinically relevant for identifying at-risk populations, guiding resource allocation, and improving culturally sensitive pain management strategies. Furthermore, establishing reliable prevalence data will support health policymakers in prioritizing early diagnosis and preventive care within dental and primary healthcare systems.

Material and methods

Building upon previously published and varied methodologies,[27–30] the first stage involved registering the project with the Open Science Framework (OSF) repository (https://osf.io/c8x2d). The project was registered on February 19, 2025, marking the official commencement of the research. The supplementary materials related to this study were made available in the OSF repository (https://osf.io/k7eds/files).

The study was conducted in accordance with the 2020 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.[31] Literature searches were performed in 2 databases: MEDLINE and Scopus. The following keyword combinations were applied:

- "orofacial pain" AND "prevalence";
- "orofacial pain" AND "epidemiology";
- "orofacial pain" AND "population";
- "facial pain" AND "prevalence";
- "facial pain" AND "epidemiology";
- "facial pain" AND "population";
- "oral pain" AND "prevalence";
- "oral pain" AND "epidemiology";
- "oral pain" AND "population".

In line with previous publications,[25,29] the analysis was restricted to studies published within the last 20 years, covering the period from January 1, 2004, to December 31, 2024.

The study design followed the PICO framework,[32,33] enabling the formulation of precise research questions and systematic data analysis:

- P (Population): individuals worldwide reporting orofacial, oral or facial pain, regardless of age or sex;
- I (Intervention): epidemiological studies assessing the prevalence of orofacial, oral or facial pain in different populations;
- C (Comparison): comparisons across geographical regions and demographic subgroups (age, sex);
- O (Outcome): estimation of the global prevalence of orofacial, oral and facial pain, and identification of potential factors influencing its distribution, particularly age and sex.

The following article types were excluded from the study: narrative reviews; systematic reviews and meta-analyses; opinion papers; case reports or case series; animal studies or biomechanical models; conference abstracts; and publications in languages other than English. Studies focusing on specific occupational or population groups (e.g., military personnel, musicians) were also excluded, unless a control group was included. In such cases, only the data from the control group was analyzed.

Grey literature was manually searched using Google, combining keywords with the names of continents, as per a previous study[25]:

- "orofacial pain" AND "North America, South America, Asia, Europe, Africa, Australia";
- "facial pain" AND "North America, South America, Asia, Europe, Africa, Australia";
- "oral pain" AND "North America, South America, Asia, Europe, Africa, Australia".

Additionally, the snowballing method was applied in accordance with Wohlin's guidelines.[34] Screening was conducted independently by 2 authors (AG and MWó) under the supervision of, and in consultation with, the first author (GZ), who had the deciding vote in cases of disagreement. The selection process was performed in 3 stages: title screening; abstract screening; and full-text assessment. It is worth emphasizing that one of the main inclusion criteria for the analysis was the presence of pain among the study participants. The flowchart of the study is presented in Fig. 1.

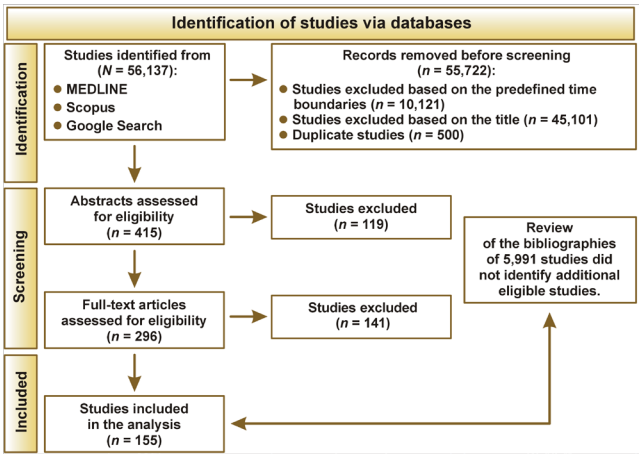

Fig. 1. Flowchart of the study selection process

Detailed information regarding excluded reports and reasons for exclusion is provided in the supplementary materials (https://osf.io/k7eds/files).

The primary database included the following variables: DOI; first author's surname; year of publication; continent where the study was conducted; participants' age; total sample size (*N*); number of females; number of males; number of individuals reporting pain; number of individuals reporting pain stratified by sex; type of pain (facial or oral); classification according to the International Classification of Orofacial Pain (ICOP)[10]; and duration of pain symptoms (in months). In accordance with previously published methodology,[24] participants were stratified into 3 age groups: <18 years; 18–60 years; and >60 years.

Due to differences in thematic scope and analytical approaches, the collected data was divided into 2 separate publications. This approach allows for a detailed discussion of each research area, ensures consistent argumentation and clear conclusions, and prevents excessive condensation of content that is essential to a comprehensive understanding of the presented findings.

In accordance with the stated aim, the study focuses on the global and continental prevalence of oral and facial pain in the general population. Information on the occurrence of oral and facial pain, classified according to the ICOP, is presented in the second publication.

The objectives and methodological descriptions of the analyzed studies were reviewed to assign them to specific categories: facial pain; oral pain; or general orofacial pain. Research papers that focused on the face were classified as facial pain, those concerning the oral cavity as oral pain, and works addressing general orofacial pain as orofacial pain. Classification was based on the assessment of the original study objectives and methodologies and was verified by the review authors.

Statistical analysis

The statistical methodology followed previously published papers.[24–26] A meta-analysis of proportions was conducted to estimate the prevalence of orofacial pain.[35] The proportion of orofacial pain cases, defined as the number of events divided by the total sample size per study, was analyzed. The logit transformation was applied to stabilize the variance of proportions and ensure that pooled estimates remained within the interval [0, 1] after back-transformation.[36]

The primary analysis employed a generalized linear mixed model (GLMM) with a logistic link function to directly model the binomial outcome of orofacial pain.[37] This method was selected for its robustness in accounting for both within-study and between-study variability without requiring continuity corrections, making it well-suited for the dataset characteristics.[38] Between-study variance (τ^2) was estimated using maximum likelihood.[36] Confidence intervals (*CI*s) for individual study proportions were constructed using the Wilson score method.[39]

A sensitivity analysis was performed using an alternative specification for the random-effects model based on the inverse variance method[40] and restricted maximum likelihood (REML) estimation,[41] which yielded comparable results with consistent heterogeneity estimates.

Subgroup analyses were conducted to explore differences in the prevalence of orofacial pain according to continent, age group and symptom duration, with separate pooled estimates calculated for each subgroup.[42] Heterogeneity within and between subgroups was assessed using the I^2 statistic and τ^2 estimates.[43] The significance of heterogeneity was evaluated using the Wald test and the likelihood ratio test (LRT). The subgroup analysis was specified to allow distinct between-study variances for each subgroup and to avoid assuming a common effect across subgroups.[42] The back-transformation option was applied to present pooled proportions on the original scale for interpretability.[36] The difference between the proportions of orofacial pain across subgroups was assessed using a Wald-type test.

Visualization of the pooled estimates, subgroup differences and heterogeneity statistics was conducted using forest plots and reported with 95% *CI*s.

Influential studies were identified using established diagnostics described by Viechtbauer and Cheung,[44] including externally standardized residuals, difference in fits (DFFITS) values, Cook's distance, covariance ratios, the leave-one-out amount of (residual) heterogeneity, the leave-one-out test statistic for (residual) heterogeneity, and differences in betas (DFBETAS).[44]

Potential publication bias was assessed using funnel plot visualization of study-specific proportion estimates against their precision (standard error (*SE*)).[45] Asymmetry in the funnel plot, which may indicate publication bias or other small-study effects, was assessed visually and statistically.

The Thompson method was applied to formally test for funnel plot asymmetry, as it provides a regression-based approach suitable for meta-analyses of proportion data.[46]

The analyses were conducted using the R statistical language (v. 4.3.3; https://www.r-project.org) on Windows 11 Pro 64 bit (build 26100), and the packages meta (v. 7.0.0; Balduzzi et al.),[47] dmetar (v. 0.1.0; Harrer et al.),[48] report (v. 0.5.8; Makowski et al.),[49] and dplyr (v. 1.1.4; Wickham et al.).[50]

Results

Due to the significant number of analyses, detailed information including the database, funnel plots with detailed descriptions, and exact *CI*s for all estimates have been included in the supplementary materials (https://osf.io/k7eds/files).

The current analysis synthesized data from 155 observational studies published between 2004 and 2024, investigating the prevalence of orofacial pain across diverse populations. After exclusion of 2 influential

studies (Bölük et al.[51] and Koopman et al.[52]), the final dataset comprised 1,712,161 participants. The mean sample size per study was 11,046 (median: 1,199; range: 42–500,488). Sex distribution was reported in 129 studies (total sample: 2,036,916), with a slight predominance of females (50.8%).

Most studies were conducted in Europe (58 studies, 945,233 participants), followed by South America (39 studies, 135,945 participants), Asia (32 studies, 96,781 participants), North America (15 studies, 415,842 participants), Africa (5 studies, 3,052 participants), and Australia (4 studies, 85,218 participants). Two studies spanned multiple continents (Asia–Europe: 1 study, 22,539 participants; North America–Australia: 1 study, 7,551 participants).

Age groups were categorized as <18 years (65 studies, 350,771 participants), 18–60 years (69 studies, 1,257,380 participants) and >60 years (11 studies, 52,206 participants). Additional categories included 18+ years (9 studies, 46,520 participants) and ≤60 years (1 study, 5,284 participants).

The reported occurrence periods for pain symptoms included 1 month (43 studies, 897,709 participants), ≥12 months (31 studies, 180,150 participants), 6 months (26 studies, 286,437 participants), and 3 months (9 studies, 236,325 participants), with 46 studies not specifying symptom duration.

The crude pooled prevalence of orofacial pain, calculated as the total number of events divided by the total sample size, was 9.53% (163,154 events). The mean study-level prevalence was 23.77% (median: 17.00%; range: 2.00–93.00%).

Concurrent oral and facial pain

A meta-analysis encompassing 155 studies, comprising 1,712,161 observations and 163,154 reported events, determined a pooled prevalence of concurrent oral and facial pain of 19.19% (95% *CI*: 16.59–22.08%). Substantial heterogeneity was observed across the studies (I^2 = 99.9%), with a τ^2 indicating considerable between-study variability and a Higgins' *H* statistic reflecting extensive diversity. The statistical tests for heterogeneity confirmed this variability as highly significant, with a Wald test *Q*-value of 136,167.87 (degrees of freedom (*df*) = 154, p = 0) and an LRT p-value of 0. Sensitivity analysis using inverse variance with REML estimation produced consistent pooled estimates and heterogeneity values, confirming the robustness of the main model. To assess potential publication bias, a funnel plot presenting the distribution of study-specific proportion estimates against their precision was generated (Fig. 2).

The analysis revealed no significant evidence of funnel plot asymmetry, with a test result of t = 0.02 (*df* = 153, p = 0.988) and a bias estimate of 0.02 (*SE* = 1.05). This minimal bias estimate supports the robustness of the pooled prevalence estimate, indicating that publication bias is unlikely to substantially influence the findings.

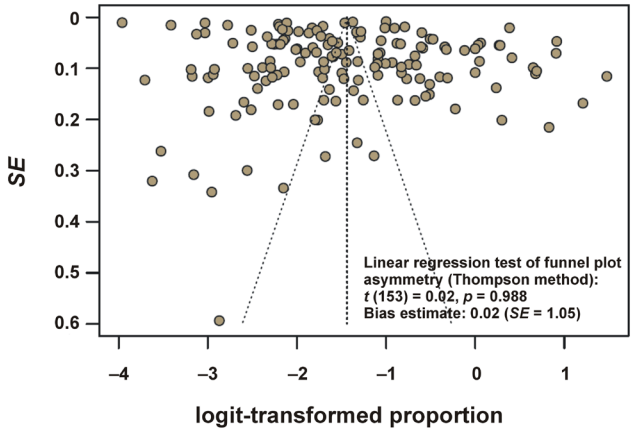

Fig. 2. Funnel plot of logit-transformed prevalence estimates with linear regression test for asymmetry (Thompson method)

SE – standard error.

Subgroup analyses by continent provided detailed insights into regional prevalence patterns, as detailed in Table 1. In Africa, the pooled prevalence was 27.44% (95% *CI*: 20.86–35.18%). Asia exhibited a higher prevalence of 30.78% (95% *CI*: 22.81–40.08%), and Australia showed a prevalence of 15.58% (95% *CI*: 11.05–21.53%). Europe reported a lower prevalence of 12.27% (95% *CI*: 9.69–15.43%), while North America had a prevalence of 14.53% (95% *CI*: 9.26–22.07%), and South America displayed a prevalence of 25.61% (95% *CI*: 20.71–31.22%).

The test for subgroup differences indicated statistically significant variation across continents, with a *Q*-value of 40.59 (*df* = 5, p < 0.0001), highlighting the influence of geographic factors on prevalence estimates. Substantial heterogeneity was observed within the subgroups, with I^2 values ranging from 88.5% in Africa to 99.9% in Europe and North America, and τ^2 values varying from 0.1530 in Africa to 1.4143 in Asia, reflecting diverse study characteristics and population differences across regions. These findings underscore that the burden of concurrent oral and facial pain is most pronounced in Asia and Africa, with prevalence rates exceeding 25%, while Europe exhibits the lowest prevalence at 12.27%.

Table 1. Summary of prevalence analysis results by region and pain type

Continent	Oral and facial pain	Facial pain	Oral pain
Africa	27	–	31
Asia	31	17	25
Australia	16	–	21
Europe	12	10	16
North America	15	16	13
South America	26	33	24
Global	19	12	21

Data presented as percentages (%). 95% confidence intervals (95% *CIs*) for all estimates are provided in the supplementary materials (https://osf.io/k7eds/files).

Subgroup analyses by age group provided detailed insights into age-specific prevalence patterns, as detailed in Table 2. The test for subgroup differences indicated statistically significant variation across age groups, with a Q-value of 15.74 (df = 2, p = 0.0004), highlighting the influence of age on prevalence estimates. Within-subgroup heterogeneity was also substantial, with I^2 values ranging from 98.8% in the >60 years group to 99.9% in the 18–60 years group, and τ^2 values varying from 1.0809 in the oldest group to 1.3368 in the youngest group, reflecting diverse study characteristics and population differences across age categories.

The above results emphasize that the burden of concurrent oral and facial pain is most pronounced among individuals aged <18 years, with a prevalence of 23.95%, followed by a decline to 16.50% in the 18–60 years group, and a further reduction to 9.83% in those older than 60 years.

Subgroup analyses by continent and age demonstrated distinct patterns in prevalence estimates (Table 2).

The test for subgroup differences indicated statistically significant variation across the 17 subgroups, with a Q-value of 253.28 (df = 16, p < 0.001), highlighting the combined influence of geographic region and age on prevalence estimates. Substantial heterogeneity was observed within the subgroups, with I^2 values ranging from 75.8% in the Africa (<18 years) subgroup to 99.9% in several subgroups, including Asia (18–60 years) and Europe (18–60 years), and τ^2 values varying from 0.0213 in the Africa (<18 years) subgroup to 2.3921 in the Asia (<18 years) subgroup, reflecting diverse study characteristics and population differences across regions and age categories. These findings indicate that the burden of concurrent oral and facial pain is most pronounced in Africa and Asia across all age groups, with prevalence exceeding 30% in several subgroups, while Europe and North America exhibit lower prevalence, particularly among older age groups.

Subgroup analyses by sex provided detailed insights into prevalence patterns. Among females, the pooled prevalence was 20.73% (95% *CI*: 16.88–25.19%), whereas among males, the prevalence was 15.20% (95% *CI*: 11.69–19.54%). The test for subgroup differences indicated marginally significant variation between sexes, with a Q-value of 3.51 (df = 1, p = 0.061), revealing a potential sex-related influence on prevalence estimates. Within-subgroup heterogeneity was substantial, with I^2 values of 99.8% for both females and males, and τ^2 values of 1.2450 for females and 1.7602 for males, reflecting diverse study characteristics and population differences across sex categories. These findings indicate a higher burden of concurrent oral and facial pain among females (20.73%) compared to males (15.20%) (Table 3).

The analysis of concurrent oral and facial pain by continent and sex is presented in Table 3. The statistical analysis indicated significant variation across the 10 subgroups, with a Q-value of 45.71 (df = 9, p < 0.001), highlighting the combined influence of geographic region and sex on prevalence estimates. Heterogeneity was substantial within the subgroups, with I^2 values ranging from 98.0% in the Australia (male) subgroup to 99.9% in the North America (male) subgroup, and τ^2 values varying from 0.15 in the Australia (male) subgroup to 2.65 in the North America (male) subgroup, reflecting diverse study characteristics and population differences across regions and sexes. These findings indicate that the burden of concurrent oral and facial pain is most pronounced in Asia and South America, particularly among females (>28%), while Europe exhibits the lowest prevalence, especially among males (8%).

Facial pain

A meta-analysis encompassing 36 studies, comprising 821,660 observations and 31,341 reported events, determined the pooled prevalence of facial pain to be 12.42% (95% *CI*: 9.17–16.62%) (Table 1). This estimate reflects a notable health burden associated with facial pain across the included studies. Substantial heterogeneity was observed across the studies, with an I^2 of 99.9% and a τ^2 indicating considerable variability between the studies. The statistical tests for heterogeneity confirmed this variability as highly significant, with a Wald test Q-value of 28,997.41

Table 2. Summary of prevalence analysis results by region and age group

Continent	Age		
	<18 years	18–60 years	>60 years
Africa	30	34*	14*
Asia	31	31	32
Australia	17*	15	–
Europe	16	12	7
North America	23	9	9
South America	26	26	1*
Global	24	16	10

Data presented as percentages (%). 95% *CIs* for all estimates are provided in the supplementary materials.
* percentage based on a single study.

Table 3. Summary of prevalence analysis results by region and sex

Continent	Females	Males
Africa	–	–
Asia	31	32
Australia	13	13
Europe	15	8
North America	18	14
South America	28	21
Global	21	15

Data presented as percentages (%). 95% *CIs* for all estimates are provided in the supplementary materials.

(df = 35, p < 0.001) and an LRT p-value <0.001. This finding indicates that facial pain affects a significant proportion of the population, with a pooled prevalence of 12.42% representing a substantial public health concern.

Subgroup analyses by continent revealed distinct regional variations in prevalence. In Asia, the pooled prevalence was 16.73% (95% *CI*: 13.67–20.30%), while Europe exhibited a lower prevalence of 10.30% (95% *CI*: 7.24–14.45%). North America showed a prevalence of 15.75% (95% *CI*: 7.37–30.53%), and South America displayed the highest prevalence at 33.44% (95% *CI*: 20.62–49.29%). The test for subgroup differences indicated statistically significant variation across the 4 continental subgroups, with a Q-value of 15.10 (df = 3, p = 0.002), highlighting the influence of geographic factors on prevalence estimates. Within-subgroup heterogeneity was substantial, with I^2 values ranging from 78.4% in Asia to 99.9% in Europe, and τ^2 values varying from 0.0229 in Asia to 1.0701 in Europe, reflecting diverse study characteristics and population differences across regions. These findings indicate that the burden of facial pain is most pronounced in South America, with a prevalence of 33.44%, while Europe exhibits the lowest prevalence at 10.30% (Table 1).

Oral pain

A meta-analysis encompassing 46 studies, comprising 400,884 observations and 65,592 reported events, determined the pooled prevalence of oral pain to be 21.04% (95% *CI*: 17.38–25.24%) (Table 1). This estimate highlights a significant health burden associated with oral pain across the included studies. Substantial heterogeneity was observed across the studies, with an I^2 of 99.8% and a τ^2 indicating considerable variability between the studies. The statistical tests for heterogeneity confirmed this variability as highly significant, with a Wald test Q-value of 22,566.93 (df = 45, p < 0.001) and an LRT p-value <0.001.

Subgroup analyses by continent revealed notable regional variations in prevalence. In Africa, the pooled prevalence was 30.79% (95% *CI*: 28.77–32.89%). Australia exhibited a prevalence of 20.61% (95% *CI*: 16.87–24.92%). Asia showed a prevalence of 24.90% (95% *CI*: 19.41–31.34%). Europe reported a prevalence of 16.49% (95% *CI*: 9.86–26.27%), and North America displayed a prevalence of 13.25% (95% *CI*: 10.19–17.05%). In South America, the prevalence was 24.22% (95% *CI*: 17.64–32.29%). The test for subgroup differences indicated statistically significant variation across the 6 continental subgroups, with a Q-value of 61.32 (df = 5, p < 0.001), highlighting the influence of geographic factors on prevalence estimates. Heterogeneity within the subgroups was substantial, with I^2 values ranging from 66.7% in Africa to 99.8% in South America, and τ^2 values varying from 0 in Africa to 0.9829 in Europe, reflecting diverse study characteristics and population differences across regions. These findings infer that the burden of oral pain is most pronounced in Africa, with a prevalence of 30.79%, followed by Asia and South America, both exceeding 24%, while North America and Europe exhibit lower prevalence rates of 13.25% and 16.49%, respectively (Table 1).

Prevalence of concurrent oral and facial pain by observation period

Subgroup analyses of the occurrence of oral and facial pain symptoms in months revealed distinct patterns. For pain reported within 1 month, the pooled prevalence was 16.19% (95% *CI*: 12.40–20.86%). For pain reported within 3 months, the prevalence was 12.06% (95% *CI*: 6.23–22.08%). For pain reported within 6 months, the prevalence was 25.30% (95% *CI*: 18.74–33.22%), while for pain reported over 12 months or more, the prevalence was 25.51% (95% *CI*: 19.80–32.20%) (Table 4). The test for subgroup differences indicated statistically significant variation across the 4 duration subgroups, with a Q-value of 11.04 (df = 3, p = 0.012), highlighting the influence of observation period on prevalence estimates. Heterogeneity within the subgroups was substantial, with I^2 values of 99.9% in the 1-month group and 99.8% in the other duration groups, and τ^2 values ranging from 0.83 in the ≥12 months group to 1.23 in the 3-month group, reflecting diverse study characteristics and population differences across duration categories. The results indicate that extending the assessment period to ≥6 months reveals a greater prevalence of concurrent oral and facial pain (>25%) compared with shorter intervals of 1–3 months (<16%).

Assessment of funnel plot asymmetry and publication bias

To evaluate potential publication bias and small-study effects, a linear regression test was conducted following the approach described by Thompson and Sharp.[46] This analysis assessed the degree of asymmetry in the funnel plots generated for the included studies. A non-significant p-value indicated insufficient evidence to reject the null hypothesis of symmetry, suggesting no substantial asymmetry in the data and, consequently, no indication of publication bias (Table 5). Funnel plots for each subgroup are provided in the supplementary materials.

Table 4. Summary of prevalence analysis results for concurrent oral and facial pain by observation period

Observation period	Studies, *n*	Prevalence [%]
1 month	43	16
3 months	9	12
6 months	26	25
≥12 months	31	26

95% *CIs* for all estimates are provided in the supplementary materials.

Table 5. Results of the linear regression test for funnel plot asymmetry using the Thompson method in meta-analyses of oral and facial pain

Analysis category	Subgroup	*t*	*df*	*p*-value	Bias statistic	*SE*
Burden of concurrent oral and facial pain by continent	Africa	−1.62	3	0.204	−4.76	2.94
	Asia	1.15	31	0.257	2.34	2.03
	Australia	−1.37	3	0.265	−14.40	10.54
	Europe	−1.51	57	0.137	−2.15	1.43
	North America	0.55	14	0.588	1.88	3.40
	South America	−0.34	37	0.738	−0.80	2.37
Global burden of concurrent oral and facial pain by age group	<18 years	1.01	64	0.317	1.76	1.75
	18–60 years	−0.12	77	0.906	−0.18	1.54
	>60 years	−0.62	18	0.542	−1.89	3.04
Burden of concurrent oral and facial pain by continent and age group	Africa: <18 years	0.58	1	0.664	1.43	2.46
	Africa: 18–60 years	not applied (1 study)				
	Africa: >60 years	not applied (1 study)				
	Asia: <18 years	1.31	12	0.215	3.74	2.86
	Asia: 18–60 years	−0.75	13	0.465	−2.93	3.89
	Asia: >60 years	3.13	2	0.089	16.62	4.35
	Australia: <18 years	not applied (1 study)				
	Australia: 18–60 years	−1.31	2	0.320	−15.34	11.69
	Australia: >60 years	not applied (0 studies)				
	Europe: <18 years	−0.46	15	0.649	−1.74	3.76
	Europe: 18–60 years	−0.59	35	0.560	−1.23	2.09
	Europe: >60 years	−1.96	8	0.086	−3.79	1.94
	North America: <18 years	0.62	5	0.560	5.62	9.01
	North America: 18–60 years	0.48	7	0.647	0.98	2.04
	North America: >60 years	−0.78	2	0.519	−2.75	3.54
	South America: <18 years	−1.15	23	0.261	−4.19	3.64
	South America: 18–60 years	0.90	12	0.388	2.26	2.52
	South America: >60 years	not applied (1 study)				
Global burden of concurrent oral and facial pain by sex	females	1.16	74	0.251	1.74	1.50
	males	−0.36	73	0.722	−0.42	1.18
Burden of concurrent oral and facial pain by continent and sex	Asia: females	−0.86	15	0.404	−2.36	2.75
	Asia: males	−0.26	15	0.799	−0.57	2.18
	Australia: females	−3.22	2	0.085	−15.46	4.81
	Australia: males	−3.54	2	0.071	−10.97	3.10
	Europe: females	0.78	30	0.439	1.88	2.40
	Europe: males	−0.76	29	0.451	−1.27	1.67
	North America: females	1.88	6	0.109	9.42	5.00
	North America: males	2.05	5	0.095	14.20	6.92
	South America: females	0.21	14	0.834	0.55	2.60
	South America: males	−0.98	15	0.342	−1.47	1.50
Global burden of facial pain	–	−0.44	34	0.662	−1.02	2.31
Global burden of oral pain	–	−0.09	44	0.926	−0.18	1.98
Burden of facial pain by continent	Africa	not applied (0 studies)				
	Asia	2.63	1	0.231	2.64	1.00
	Australia	not applied (0 studies)				
	Europe	−0.88	26	0.386	−2.26	2.56
	North America	1.23	1	0.435	29.00	23.62
	South America	−6.64	1	0.095	−9.60	1.45
Burden of oral pain by continent	Africa	not applied (2 studies)				
	Asia	0.51	6	0.626	2.16	4.21
	Australia	0.06	1	0.965	0.40	7.22
	Europe	−0.47	9	0.650	−1.60	3.41
	North America	−0.39	3	0.726	−2.17	5.63
	South America	−0.23	16	0.818	−0.87	3.74
Concurrent oral and facial pain by duration	1 month	−0.42	41	0.676	−0.98	2.33
	3 months	0.56	7	0.593	4.92	8.80
	6 months	1.83	23	0.081	5.71	3.12
	≥12 months	0.89	28	0.379	3.20	3.58

t – regression test statistic; *df* – degrees of freedom; *p*-value – probability of observing the results under the null hypothesis of no asymmetry; bias statistic – estimate of the intercept or slope indicating bias; *SE* – standard error.

Discussion

The primary aim of this study was to determine the global prevalence of oral and facial pain in the general population and across continents. The results confirmed the initial hypothesis, demonstrating that the global prevalence of oral and facial pain remains below 30%, supporting the assumption of a moderate but clinically significant worldwide burden. To begin this discussion, it is important to emphasize that the analysis encompassed 155 studies and over 1.7 million observations. Attention should be drawn to the scale of the problem of comorbid oral and facial pain, as well as to the considerable variability in findings depending on continent, age, sex, and symptom duration. The estimated global prevalence of approx. 19% indicates a notable health burden that extends beyond the local context and should be regarded as a significant public health concern. However, the analyzed results demonstrated a high degree of heterogeneity, which profoundly influences the interpretation of these outcomes and reflects the multidimensional complexity of orofacial pain phenomena.

High heterogeneity (I^2 values exceeding conventional thresholds) indicates that the included studies differ substantially in design, population characteristics and diagnostic definitions. From a methodological standpoint, this heterogeneity reduces the precision of pooled estimates, suggesting that the global prevalence should be interpreted as a broad epidemiological tendency rather than an exact quantitative measure.[43,46,53] Clinically, it highlights the contextual dependence of orofacial pain, the expression and reporting of which vary according to cultural, socioeconomic and healthcare system factors. Therefore, while heterogeneity limits the statistical generalizability of the results, it simultaneously enriches understanding by revealing the complexity of pain as a biopsychosocial construct.

When comparing our findings with previous epidemiological studies, it is worth recalling data from the last century, which estimated that orofacial pain occurred in approx. 10% of the adult population.[54,55] The observed increase over the past 25 years may have multiple causes, ranging from improved diagnostics and greater awareness of the problem among both patients and clinicians to lifestyle changes, stress, an increasing number of risk factors, and the residual effects of the coronavirus disease 2019 (COVID-19) pandemic.[11,26,56–58] The COVID-19 pandemic likely influenced pain perception and prevalence indirectly through increased psychosocial stress, changes in access to dental and medical care, and altered health-seeking behaviors. Lockdowns and healthcare disruptions may have contributed to delayed diagnoses, while elevated anxiety and bruxism during the pandemic period could have temporarily increased the prevalence of orofacial pain in certain populations.[18,25,59]

When analyzed by continent, a particularly striking contrast emerges between Asia and Africa, where prevalence rates exceed 27–30%, and Europe or North America, where the rates are approx. 12% and 14.5%, respectively. This suggests that geographic factors, including cultural, economic and biological determinants, play a key role in shaping the distribution of such conditions. These findings have been underscored in recent (2024–2025) meta-analyses and forecasts.[24–27] However, it is important to recognize that such substantial intercontinental variation reflects not only true epidemiological differences but also methodological heterogeneity among studies. Differences in sampling strategies, diagnostic criteria, data collection methods, and reporting standards may contribute to variability in prevalence estimates. Therefore, heterogeneity in this context should not be treated merely as statistical noise, but rather as an expression of the inherent variability in pain-related experiences across human populations.

It can be assumed that these differences arise from variations in dental care patterns, access to pain management, dietary habits, and psychosocial determinants such as stress and socioeconomic status.[24,60] Cultural norms also influence responses to pain: in some societies, open expression of suffering is socially acceptable, while in others, restraint is emphasized.[60–62] Such cultural frameworks shape not only the interpretation and reporting of pain, but also coping strategies.[60,63,64]

A similar pattern of heterogeneity is evident across age groups. The highest prevalence was observed among individuals under 18 years of age (23.95%), suggesting that children and adolescents may be particularly vulnerable to such conditions. Contributing factors include frequent orthodontic problems, dental caries and psychosocial determinants such as greater susceptibility to academic stress and challenges associated with developmental changes.[65–67] By contrast, older adults (>60 years) showed a markedly lower prevalence of pain (9.83%). This paradoxical trend warrants deeper analysis. One possible explanation involves neurobiological adaptations to chronic nociceptive stimuli, leading to altered pain processing (hypoalgesia) with advancing age.[68–71] Furthermore, age-related degeneration of peripheral nociceptors, the use of analgesic or anti-inflammatory medications, and potential underreporting due to reduced pain awareness or social normalization of discomfort in older age may contribute to this phenomenon.[68–71] Psychologically, older adults may also demonstrate greater emotional regulation and coping efficiency, diminishing perceived pain intensity. Thus, the age-related paradox likely reflects a complex interplay between biological desensitization, pharmacological influences and sociocognitive factors.[70,72,73]

Sex-based differences provide another dimension of variability. Women reported orofacial pain more frequently than men (20.73% vs. 15.20%), consistent with broader observations in pain research. The higher prevalence of pain among women may be partially explained by hormonal influences, as female hormones have been shown to modulate pain perception.[20,21] A greater burden of orofacial pain in women is a well-documented finding

in the literature.[24,74] Nevertheless, the magnitude of sex differences varies substantially across studies, reflecting heterogeneity in methodologies and populations.

Separating oral pain from facial pain yielded additional insights. Oral pain (21.04%) was more prevalent than facial pain (12.42%), suggesting that dental and periodontal conditions are primary drivers of the problem. Marked regional differences are also apparent: in Africa, 30.79% of individuals experience oral pain, compared with 13.25% in North America. The highest prevalence of facial pain was observed in South America (33.44%), once again highlighting the influence of environmental, economic and healthcare access factors. However, caution is warranted when interpreting these results, as heterogeneity in study quality and pain definitions could partially inflate apparent differences.

Analyses focusing on Africa and Australia are pioneering, as earlier meta-analyses highlighted significant gaps in the literature for these regions.[24,25] Notably, the reported prevalence of oral pain in Africa (30.79%) closely aligns with projected rates of TMD for the continent in 2030, estimated at 37% (95% *CI*: 32–42%).[27] In the South American context (facial pain prevalence of 33.44%), it is relevant to compare these findings with the results of a 2025 meta-regression showing that in North America, comorbidity between bruxism and TMD reached 70%, with a mean TMD prevalence of 98.3% among patients with bruxism.[26] These observations underscore the urgent need for further research and preventive measures on these continents.

At the beginning of this century (2002), it was reported that the prevalence of orofacial pain was 7% over a 7-day period and 16% over a 12-month period.[54] Likewise, our analysis demonstrated an increasing trend in pain prevalence with longer assessment windows, although current global prevalence rates are higher. Short observation periods yielded lower prevalence estimates (16.19% for up to 1 month and 12.06% for up to 3 months), whereas prevalence exceeded 25% when pain was assessed for 6 months or longer.[54,75,76]

These findings have several implications for public health and clinical practice. First, in terms of healthcare resource allocation, regions with high prevalence of orofacial pain (e.g., Africa, South America and Asia) should prioritize expanding access to dental and pain management services. Second, regarding screening recommendations, standardized pain assessment tools should be incorporated into routine dental and primary care visits to facilitate early detection. Third, preventive strategies should emphasize education on oral hygiene, stress reduction and early management of temporomandibular dysfunctions. Collectively, these approaches may improve global oral health outcomes and reduce the long-term burden of orofacial pain.

The primary limitation of the present study is the high heterogeneity among the included studies, which reduces the precision of pooled estimates. The database was created based on published data provided by the original study authors, which introduces a potential risk of reporting bias; however, this approach is commonly accepted in meta-analytic research.[24,25] Furthermore, the study did not account for additional covariates such as local pain response, healthcare quality or the reliability of self-assessment.

Another important limitation is that the vast majority of included studies relied on self-reported pain assessment tools, the validity and comparability of which remain limited. Instruments such as the visual analogue scale (VAS), numeric rating scale (NRS) or various pain questionnaires are not equivalent either in what they measure (e.g., intensity, frequency, presence of pain, or impact on daily functioning) or in how participants interpret the questions. The diversity of applied tools can lead to systematic differences in reporting. Some scales tend to produce higher pain scores, while others may underestimate intensity.[77,78] This lack of standardization significantly complicates cross-study comparisons and increases the risk of measurement error, which in turn may contribute to over- or underestimation of the true global prevalence of orofacial pain.

It should also be emphasized that self-reported pain is susceptible to both recall bias and cultural differences in pain expression. Participants often report pain retrospectively, based on general impressions rather than precise recollection of pain episodes, which can lead to over-reporting among individuals with chronic pain and under-reporting in those with episodic pain. Additionally, cultural norms regarding the acceptability of expressing discomfort, displaying suffering, or expectations related to healthcare interactions may shape self-reports. In some cultures, minimizing complaints is more typical, while in others, detailed and open reporting of pain is normative.[60–64] These cultural factors, combined with the inherent subjectivity of self-reports, may result in substantial population-level differences that do not necessarily reflect actual prevalence but rather distinct styles of communication and perception of discomfort.

In the current study, a formal risk-of-bias assessment at the level of individual studies was not conducted,[79,80] as the analysis was designed as a proportion meta-analysis primarily based on statistical methodology.

In summary, the present meta-analysis provides compelling evidence of a substantial population-level burden associated with oral and facial pain, while also demonstrating that this phenomenon is strongly modulated by age, sex, geographic region, and symptom duration. Crucially, the considerable heterogeneity observed across studies should not be seen merely as a methodological challenge but rather as a reflection of the complex, context-dependent nature of orofacial pain. Understanding this variability offers valuable insights into underlying biological, social and cultural mechanisms. Thus, although heterogeneity complicates straightforward interpretation,

it enriches the discussion and underscores the need for standardized research approaches and context-sensitive prevention strategies.

Conclusions

1. The present study indicates that the global prevalence is 19% for concurrent oral and facial pain, 12% for facial pain and 21% for oral pain.
2. A relatively high prevalence of oral pain (31%) and combined oral and facial pain (27%) was observed in Africa. Further research on facial pain in this region is recommended. These findings infer that the burden of oral pain is particularly pronounced in Africa.
3. A prevalence of 31% for concurrent oral and facial pain was observed in Asia, with 17% experiencing facial pain and 21% oral pain.
4. Australia exhibited a lower overall prevalence of concurrent oral and facial pain (16%), but the prevalence of oral pain was notably higher (25%). Due to the limited number of studies available, these results should be considered preliminary and interpreted with caution. It is recommended that further research on facial pain be conducted in this region.
5. Comparatively low prevalence rates were observed in Europe across all categories: 12% for concurrent oral and facial pain; 10% for facial pain; and 16% for oral pain. These findings indicate that facial pain has the lowest prevalence in Europe.
6. North America presented moderate and consistent levels, with 15% experiencing concurrent oral and facial pain, 16% experiencing facial pain and 13% experiencing oral pain. These results suggest that oral pain has the lowest prevalence in North America.
7. The highest recorded prevalence of facial pain (33%) was observed in South America; in this region, the prevalence of oral pain was 24% and that of concurrent oral and facial pain was 26%. These findings indicate that the burden of facial pain may be most pronounced in South America.
8. Concurrent oral and facial pain was most prevalent in individuals up to 18 years of age (24%). This prevalence decreased to 16% among those aged 18–60 years and further declined to 10% among individuals aged >60 years, highlighting a clear age-related downward trend.
9. When considering both continent and age group, concurrent oral and facial pain was most prevalent in Africa and Asia, where rates exceeded 30% in several subgroups. In contrast, Europe and North America exhibited lower prevalence levels, particularly among older age groups.
10. Females demonstrated a higher prevalence of concurrent oral and facial pain (21%) compared with males (15%). Overall, the burden of concurrent oral and facial pain was highest in Asia and South America, particularly among females (exceeding 28%), whereas the lowest prevalence was observed in Europe, especially among males (8%).
11. The occurrence of pain symptoms across different observation periods is as follows: within 1 month, 16% of the population experienced pain; within 3 months, 12%; within 6 months, 25%; and within 12 months or longer, 26%. These findings indicate that the prevalence of concurrent oral and facial pain is most pronounced for observation periods of 6 months and longer, exceeding 25%, while shorter durations (1 and 3 months) show lower prevalence.
12. The results of the present study should be interpreted with caution due to the high heterogeneity among the included studies. Further epidemiological research is recommended, employing rigorous methodology and adherence to current pain classification systems.

Ethics approval and consent to participate

Not applicable.

Data availability

The data related to this article, including supplementary materials, is available in the Open Science Framework database (https://osf.io/k7eds/files). The script used in the analysis is available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Belfer I. Nature and nurture of human pain. *Scientifica (Cairo)*. 2013;2013:415279. doi:10.1155/2013/415279
2. Gozzi N, Preatoni G, Ciotti F, et al. Unraveling the physiological and psychosocial signatures of pain by machine learning. *Med*. 2024;5(12):1495.e5–1509.e5. doi:10.1016/j.medj.2024.07.016
3. Raja SN, Carr DB, Cohen M, et al. The revised International Association for the Study of Pain definition of pain: Concepts, challenges, and compromises. *Pain*. 2020;161(9):1976–1982. doi:10.1097/j.pain.0000000000001939
4. Garland EL. Pain processing in the human nervous system: A selective review of nociceptive and biobehavioral pathways. *Prim Care*. 2012;39(3):561–571. doi:10.1016/j.pop.2012.06.013

5. Scribante A, Pellegrini M, Pulicari F, Ghizzoni M, Modugno FP, Spadari F. Pain assessment in oral medicine through its different dimensions: A comprehensive review. *Dent J (Basel)*. 2023;11(11):246. doi:10.3390/dj11110246
6. Romero-Reyes M, Uyanik JM. Orofacial pain management: Current perspectives. *J Pain Res*. 2014;7:99–115. doi:10.2147/JPR.S37593
7. Val M, Manfredini D, Guarda Nardini L. Is botulinum toxin the future of orofacial pain management? Evidence and perspectives. *Dent Med Probl*. 2025;62(3):405–407. doi:10.17219/dmp/200127
8. Zieliński G, Gawda P. Defining effect size standards in temporomandibular joint and masticatory muscle research. *Med Sci Monit*. 2025;31:e948365. doi:10.12659/MSM.948365
9. International Association for the Study of Pain (IASP). Orofacial Pain. https://www.iasp-pain.org/advocacy/global-year/orofacial-pain. Accessed August 11, 2025.
10. International Classification of Orofacial Pain, 1st edition (ICOP), 2020. *Cephalalgia*. 2020;40(2):129–221. doi:10.1177/0333102419893823
11. Wieckiewicz M, Jenca A, Seweryn P, et al. Determination of pain intensity, pain-related disability, anxiety, depression, and perceived stress in Polish adults with temporomandibular disorders: A prospective cohort study. *Front Integr Neurosci*. 2022;16:1026781. doi:10.3389/fnint.2022.1026781
12. Seweryn P, Waliszewska-Prosol M, Petrasova A, et al. Central sensitisation, anxiety and depressive symptoms in patients with chronic masticatory muscle pain. *J Oral Rehabil*. 2025;52(11):2010–2020. doi:10.1111/joor.70007
13. Tao X, Cheng C, Bai J. Association between chronic pain, coping strategies, and sleep quality in rural chinese older adults: A cross-sectional study. *Clin Interv Aging*. 2025;20:1005–1014. doi:10.2147/CIA.S533869
14. Sessle BJ. Chronic orofacial pain: Models, mechanisms, and genetic and related environmental influences. *Int J Mol Sci*. 2021;22(13):7112. doi:10.3390/ijms22137112
15. Morales ME, Yong RJ. Racial and ethnic disparities in the treatment of chronic pain. *Pain Med*. 2021;22(1):75–90. doi:10.1093/pm/pnaa427
16. Meints SM, Cortes A, Morais CA, Edwards RR. Racial and ethnic differences in the experience and treatment of noncancer pain. *Pain Manag*. 2019;9(3):317–334. doi:10.2217/pmt-2018-0030
17. Kim HJ, Yang GS, Greenspan JD, et al. Racial and ethnic differences in experimental pain sensitivity: Systematic review and meta-analysis. *Pain*. 2017;158(2):194–211. doi:10.1097/j.pain.0000000000000731
18. Orzeszek S, Martynowicz H, Smardz J, et al. Relationship between pain, quality of sleep, sleep bruxism and patients' personality among individuals with reported orofacial pain. *Sci Rep*. 2025;15(1):25009. doi:10.1038/s41598-025-09978-w
19. Orzeszek S, Martynowicz H, Smardz J, et al. Assessment of sleep quality in patients with orofacial pain and headache complaints: A polysomnographic study. *Dent Med Probl*. 2024;61(4):549–562. doi:10.17219/dmp/177008
20. Berger M, Szalewski L, Bakalczuk M, Bakalczuk G, Bakalczuk S, Szkutnik J. Association between estrogen levels and temporomandibular disorders: A systematic literature review. *Prz Menopauzalny*. 2015;14(4):260–270. doi:10.5114/pm.2015.56538
21. Zieliński G, Pająk-Zielińska B. Association between estrogen levels and temporomandibular disorders: An updated systematic review. *Int J Mol Sci*. 2024;25(18):9867. doi:10.3390/ijms25189867
22. Porporatti AL, Schroder ÂGD, Lebel A, et al. Prevalence of orofacial and head pain: An umbrella review of systematic reviews. *J Oral Facial Pain Headache*. 2024;38(3):1–14. doi:10.22514/jofph.2024.022
23. Macedo AC, Bitencourt FV, de Faria AOV, et al. Prevalence of orofacial pain in individuals with mild cognitive impairment or dementia: A systematic review and meta-analysis. *Gerodontology*. 2024;41(3):335–345. doi:10.1111/ger.12740
24. Zieliński G, Pająk-Zielińska B, Ginszt M. A meta-analysis of the global prevalence of temporomandibular disorders. *J Clin Med*. 2024;13(5):1365. doi:10.3390/jcm13051365
25. Zieliński G, Pająk A, Wójcicki M. Global prevalence of sleep bruxism and awake bruxism in pediatric and adult populations: A systematic review and meta-analysis. *J Clin Med*. 2024;13(14):4259. doi:10.3390/jcm13144259
26. Zieliński G, Pająk-Zielińska B, Pająk A, Wójcicki M, Litko-Rola M, Ginszt M. Global co-occurrence of bruxism and temporomandibular disorders: A meta-regression analysis. *Dent Med Probl*. 2025;62(2):309–321. doi:10.17219/dmp/201376
27. Zieliński G. Quo vadis temporomandibular disorders? By 2050, the global prevalence of TMD may approach 44%. *J Clin Med*. 2025;14(13):4414. doi:10.3390/jcm14134414
28. Kolpashnikova K, Desai S. Fear of falling: Scoping review and topic analysis protocol. *BMJ Open*. 2023;13(2):e066652. doi:10.1136/bmjopen-2022-066652
29. Zieliński G. Effect size guidelines for individual and group differences in physiotherapy. *Arch Phys Med Rehabil*. 2025:106(12):1844–1849. doi:10.1016/j.apmr.2025.05.013
30. Huang X, Zhu D, Cao Y, Li W, Lai J, Ren Y. Protocol for a systematic review and meta-analysis of recurrence and metastasis of different surgical techniques for non-small cell lung cancer. *BMJ Open*. 2024;14(8):e086503. doi:10.1136/bmjopen-2024-086503
31. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71
32. Brown D. A review of the PubMed PICO Tool: Using evidence-based practice in health education. *Health Promot Pract*. 2020;21(4):496–498. doi:10.1177/1524839919893361
33. Frandsen TF, Bruun Nielsen MF, Lindhardt CL, Eriksen MB. Using the full PICO model as a search tool for systematic reviews resulted in lower recall for some PICO elements. *J Clin Epidemiol*. 2020;127:69–75. doi:10.1016/j.jclinepi.2020.07.005
34. Wohlin C. Guidelines for snowballing in systematic literature studies and a replication in software engineering. In: *Proceedings of the 18th International Conference on Evaluation and Assessment in Software Engineering*. New York, NY: Association for Computing Machinery; 2014:1–10. doi:10.1145/2601248.2601268
35. Schwarzer G, Chemaitelly H, Abu-Raddad LJ, Rücker G. Seriously misleading results using inverse of Freeman–Tukey double arcsine transformation in meta-analysis of single proportions. *Res Synth Methods*. 2019;10(3):476–483. doi:10.1002/jrsm.1348
36. Barendregt JJ, Doi SA, Lee YY, Norman RE, Vos T. Meta-analysis of prevalence. *J Epidemiol Community Health*. 2013;67(11):974–978. doi:10.1136/jech-2013-203104
37. Stijnen T, Hamza TH, Ozdemir P. Random effects meta-analysis of event outcome in the framework of the generalized linear mixed model with applications in sparse data. *Stat Med*. 2010;29(29):3046–3067. doi:10.1002/sim.4040
38. Lin L, Chu H. Meta-analysis of proportions using generalized linear mixed models. *Epidemiology*. 2020;31(5):713–717. doi:10.1097/EDE.0000000000001232
39. Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. *Am Stat*. 1998;52(2):119–126. doi:10.2307/2685469
40. DerSimonian R, Laird N. Meta-analysis in clinical trials. *Control Clin Trials*. 1986;7(3):177–188. doi:10.1016/0197-2456(86)90046-2
41. Viechtbauer W. Bias and efficiency of meta-analytic variance estimators in the random-effects model. *J Educ Behav Stat*. 2005;30(3):261–293. doi:10.3102/10769986030003261
42. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. A basic introduction to fixed-effect and random-effects models for meta-analysis. *Res Synth Methods*. 2010;1(2):97–111. doi:10.1002/jrsm.12
43. Higgins JPT, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med*. 2002;21(11):1539–1558. doi:10.1002/sim.1186
44. Viechtbauer W, Cheung MWL. Outlier and influence diagnostics for meta-analysis. *Res Synth Methods*. 2010;1(2):112–125. doi:10.1002/jrsm.11
45. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ*. 1997;315(7109):629–634. doi:10.1136/bmj.315.7109.629
46. Thompson SG, Sharp SJ. Explaining heterogeneity in meta-analysis: A comparison of methods. *Stat Med*. 1999;18(20):2693–2708. doi:10.1002/(sici)1097-0258(19991030)18:20<2693::aid-sim235>3.0.co;2-v
47. Balduzzi S, Rücker G, Schwarzer G. How to perform a meta-analysis with R: A practical tutorial. *Evid Based Ment Health*. 2019;22(4):153–160. doi:10.1136/ebmental-2019-300117
48. Harrer M, Cuijpers P, Furukawa T, Ebert DD. dmetar: Companion R Package For The Guide 'Doing Meta-Analysis in R (R package version 0.1.0). 2019. https://dmetar.protectlab.org. Accessed November 16, 2023.

49. Makowski D, Lüdecke D, Patil I, Thériault R, Ben-Shachar M, Wiernik B. Automated results reporting as a practical tool to improve reproducibility and methodological best practices adoption. CRAN. 2023. https://easystats.github.io/report. Accessed November 16, 2023.
50. Wickham H, François R, Henry L, Müller K, Vaughan D, Posit Software. dplyr: A Grammar of Data Manipulation. https://cran.r-project.org/web/packages/dplyr/index.html. Accessed November 16, 2023.
51. Bölük C, Türk Börü Ü, Taşdemir M. The prevalence of trigeminal neuralgia in Turkey: A population-based study. *Neurol Res*. 2020;42(11):968–972. doi:10.1080/01616412.2020.1794372
52. Koopman JSHA, Dieleman JP, Huygen FJ, de Mos M, Martin CGM, Sturkenboom MCJM. Incidence of facial pain in the general population. *Pain*. 2009;147(1–3):122–127. doi:10.1016/j.pain.2009.08.023
53. Migliavaca CB, Stein C, Colpani V, et al.; Prevalence Estimates Reviews-Systematic Review Methodology Group (PERSyst). Meta-analysis of prevalence: I^2 statistic and how to deal with heterogeneity. *Res Synth Methods*. 2022;13(3):363–367. doi:10.1002/jrsm.1547
54. Kohlmann T. Epidemiology of orofacial pain [in German]. *Schmerz*. 2002;16(5):339–345. doi:10.1007/s004820200000
55. LeResche L. Epidemiology of temporomandibular disorders: Implications for the investigation of etiologic factors. *Crit Rev Oral Biol Med*. 1997;8(3):291–305. doi:10.1177/10454411970080030401
56. Yanez Regonesi F, Kaspo GA, Boggero IA, Moreno-Hay I. The impact of the COVID-19 pandemic on orofacial pain practice: Perceptions from a convenience sample of orofacial pain practitioners. *J Am Dent Assoc*. 2023;154(3):266–271. doi:10.1016/j.adaj.2022.03.012
57. Zhuang S, Li H, Lin Y, et al. The effect of COVID-19 infection on orofacial pain: A cross-sectional study. *Int Dent J*. 2025;75(2):514–523. doi:10.1016/j.identj.2024.07.007
58. Seweryn P, Orzeszek SM, Waliszewska-Prosół M, et al. Relationship between pain severity, satisfaction with life and the quality of sleep in Polish adults with temporomandibular disorders. *Dent Med Probl*. 2023;60(4):609–617. doi:10.17219/dmp/171894
59. do Carmo Cerqueira TR, Batista SG, de Mello EB, DosSantos MF, de Campos Tuñas IT. Impact of the COVID-19 pandemic on stress, sleep, and oral health in university students. *Front Pain Res (Lausanne)*. 2021;2:744264. doi:10.3389/fpain.2021.744264
60. Peacock S, Patel S. Cultural influences on pain. *Rev Pain*. 2008;1(2):6–9. doi:10.1177/204946370800100203
61. Atkins D, Uskul AK, Cooper NR. Culture shapes empathic responses to physical and social pain. *Emotion*. 2016;16(5):587–601. doi:10.1037/emo0000162
62. Bhojwani M, Walimbe V, Malani R. Pain neuroscience education through cultural lens: Insights, challenges and future implications. *J Man Manip Ther*. 2024;32(2):127–130. doi:10.1080/10669817.2024.2317513
63. Reis FJJ, Nijs J, Parker R, Sharma S, Wideman TH. Culture and musculoskeletal pain: Strategies, challenges, and future directions to develop culturally sensitive physical therapy care. *Braz J Phys Ther*. 2022;26(5):100442. doi:10.1016/j.bjpt.2022.100442
64. Rogger R, Bello C, Romero CS, Urman RD, Luedi MM, Filipovic MG. Cultural framing and the impact on acute pain and pain services. *Curr Pain Headache Rep*. 2023;27(9):429–436. doi:10.1007/s11916-023-01125-2
65. Lin W, Farella M, Antoun JS, Topless RK, Merriman TR, Michelotti A. Factors associated with orthodontic pain. *J Oral Rehabil*. 2021;48(10):1135–1143. doi:10.1111/joor.13227
66. Ribeiro Junior CA, Vettore MV, Rebelo Vieira JM, et al. The role of dental pain and psychosocial factors on the relationship between dental caries and oral health-related quality of life in children. *BMC Oral Health*. 2022;22(1):340. doi:10.1186/s12903-022-02372-2
67. Jastrowski Mano KE. School anxiety in children and adolescents with chronic pain. *Pain Res Manag*. 2017;2017:8328174. doi:10.1155/2017/8328174
68. Domenichiello AF, Ramsden CE. The silent epidemic of chronic pain in older adults. *Prog Neuropsychopharmacol Biol Psychiatry*. 2019;93:284–290. doi:10.1016/j.pnpbp.2019.04.006
69. Mullins S, Hosseini F, Gibson W, Thake M. Physiological changes from ageing regarding pain perception and its impact on pain management for older adults. *Clin Med (Lond)*. 2022;22(4):307–310. doi:10.7861/clinmed.22.4.phys
70. Tinnirello A, Mazzoleni S, Santi C. Chronic pain in the elderly: Mechanisms and distinctive features. *Biomolecules*. 2021;11(8):1256. doi:10.3390/biom11081256
71. Fernández-Palacios FG, Tejera-Alonso A, Pacho-Hernández JC, et al. Effects of aging on experimentally induced pain perception during a distraction task. *Sci Rep*. 2025;15(1):10574. doi:10.1038/s41598-025-94849-7
72. Boggero IA, Geiger PJ, Segerstrom SC, Carlson CR. Pain intensity moderates the relationship between age and pain interference in chronic orofacial pain patients. *Exp Aging Res*. 2015;41(4):463–474. doi:10.1080/0361073X.2015.1053770
73. Ghandehari O, Gallant NL, Hadjistavropoulos T, Williams J, Clark DA. The relationship between the pain experience and emotion regulation in older adults. *Pain Med*. 2020;21(12):3366–3376. doi:10.1093/pm/pnaa135
74. Lövgren A, Vallin S, Häggman-Henrikson B, et al. Women are worse off in developing and recovering from temporomandibular disorder symptoms. *Sci Rep*. 2025;15(1):4732. doi:10.1038/s41598-025-86502-0
75. Lovette BC, Bannon SM, Spyropoulos DC, Vranceanu AM, Greenberg J. "I still suffer every second of every day": A qualitative analysis of the challenges of living with chronic orofacial pain. *J Pain Res*. 2022;15:2139–2148. doi:10.2147/JPR.S372469
76. Durham J, Shen J, Breckons M, et al. Healthcare cost and impact of persistent orofacial pain: The DEEP study cohort. *J Dent Res*. 2016;95(10):1147–1154. doi:10.1177/0022034516648088
77. Herr KA, Garand L. Assessment and measurement of pain in older adults. *Clin Geriatr Med*. 2001;17(3):457–478. doi:10.1016/s0749-0690(05)70080-x
78. Karcioglu O, Topacoglu H, Dikme O, Dikme O. A systematic review of the pain scales in adults: Which to use? *Am J Emerg Med*. 2018;36(4):707–714. doi:10.1016/j.ajem.2018.01.008
79. Hoy D, Brooks P, Woolf A, et al. Assessing risk of bias in prevalence studies: Modification of an existing tool and evidence of interrater agreement. *J Clin Epidemiol*. 2012;65(9):934–939. doi:10.1016/j.jclinepi.2011.11.014
80. Ma LL, Wang YY, Yang ZH, Huang D, Weng H, Zeng XT. Methodological quality (risk of bias) assessment tools for primary and secondary medical studies: What are they and which is better? *Mil Med Res*. 2020;7(1):7. doi:10.1186/s40779-020-00238-8

Review

Role of curcumin and its nanoformulations in the management of oral squamous cell carcinoma: A systematic review

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Abstract

Indian spice curcumin, which has anti-tumor, anti-inflammatory and antioxidant effects, has showed its potential as an innovative adjunct for the treatment of oral squamous cell cancer (OSCC), as well as an intriguing chemopreventive drug. The goal of this review was to consolidate the salient characteristics of curcumin and its cutting-edge nanoformulations, and to further outline the role of curcumin in the management of OSCC.

The PubMed, Scopus and Cochrane databases were used to search for evidence-based research papers on curcumin.

The current systematic review included 35 publications. There were 5 clinical studies and 30 cell line studies. The included studies employed a wide range of OSCC cell lines, with CAL-27 in 6 studies, KB in 4 studies, FaDu in 3 studies, and SCC-9 in 3 studies, being the most common. Each of the entailed study found that when cell lines were treated with curcumin, there was an overall decrease in the proliferation of cells and cell growth when measured by the MTT assay, the luciferase assay and immunofluorescence. In clinical studies, APG-157 could inhibit tumor cell death by lowering the concentrations of NF-κB-driven cytokines that induce inflammation. The WCRF International/UoB framework-recommended quality assessment of cell line studies regarded 6 studies as high-quality and 3 studies were deemed of moderate quality.

The novel formulations of curcumin have been explored for its usefulness in the management of oral cancer, with promising results.

Keywords: apoptosis, nanoformulations, curcumin, oral squamous cell carcinoma, demethoxycurcumin

Highlights

- Curcumin exhibits significant anticancer activity in oral squamous cell carcinoma (OSCC) by inhibiting cell proliferation, inducing apoptosis via intrinsic pathways, and causing G2/M phase cell cycle arrest.
- Nanoformulation-based delivery systems enhance the bioavailability, stability and intracellular uptake of curcumin, improving its therapeutic potential in oral cancer (OC) treatment.
- The combination therapies involving curcumin nanoformulations can increase intracellular drug concentration and produce synergistic anti-cancer effects.
- Clinical and experimental studies suggest that curcumin may reduce tumor size and exert anti-inflammatory and cytotoxic effects in OC.
- Further research is required to clarify the bio-interactions between curcumin nanoparticles and cellular structures, and to optimize advanced delivery systems, such as liposomes and polymeric nanoparticles.

Introduction

Indian spice curcumin, which has anti-tumor, anti-inflammatory and antioxidant effects, has showed its potential as an innovative adjunct for the treatment of oral squamous cell cancer (OSCC), as well as an intriguing chemopreventive drug.[1] Curcumin has demonstrated its effectiveness against a variety of chronic conditions, including tumors, neurological illnesses, hepatic impairment, obesity-related disorders, and arthritic conditions. As stand-alone treatment or in conjunction with different therapies, curcumin is exceptionally effective in the management of cancer. Additionally, this organic substance has the ability to modulate a number of cellular receptors and signaling pathways connected with multiple cancers.[2]

Advanced-stage OSCC cells develop into tumors that are highly resistant to chemotherapeutic agents due to their ability to alter key biological processes. These adaptations can lead to therapeutic failure, including ineffective drug delivery to target sites, metabolic reprogramming, altered redox balance, and impaired DNA damage repair mechanisms. Over the past several decades, extensive research has focused on developing innovative strategies to treat cancer cells that are resistant to multiple therapies by blocking resistance pathways and employing modern anti-cancer agents. Curcumin, a naturally occurring compound, has attracted considerable attention, since structural modifications have enabled the manufacturing of analogs and derivatives with enhanced anti-cancer activity and relatively low adverse effects, contributing to the development of novel anti-tumor therapeutics.[3]

Curcumin exhibits a multifaceted anti-cancer mechanism of action by modulating numerous molecular pathways.[4] It inhibits several components involved in cellular proliferation, including mitogen-activated protein kinases (MAPKs), c-Jun N-terminal kinases (JNKs) and cyclin-dependent kinases (CDKs), which are frequently overexpressed in tumor cells. Curcumin also reduces the expression of growth factor receptors, such as insulin-like growth factor (IGF), fibroblast growth factor (FGF) and epidermal growth factor (EGF). In addition, curcumin promotes apoptosis by upregulating the pro-apoptotic protein p53 while suppressing anti-apoptotic signaling molecules, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), B-cell lymphoma 2 (Bcl-2), signal transducer and activator of transcription 3 (STAT3), and survivin. Its anti-angiogenic effects are attributed to its ability to inhibit the expression of pro-angiogenic factors, particularly hypoxia-inducible factor 1 (HIF-1), vascular endothelial growth factor (VEGF), and angiopoietin 1 (Ang-1). Curcumin also modulates the enzymes and proteins involved in tumor invasion and metastasis, such as transforming growth factor beta (TGF-β), matrix metalloproteinases (MMPs), cell adhesion molecules (CAMs), tissue plasminogen activator (tPA), and lysyl oxidase (LOX). Furthermore, curcumin can regulate the expression of microRNAs associated with tumor initiation, growth and progression. It exerts anti-inflammatory effects by decreasing the production of pro-inflammatory cytokines, including interleukin 1 (IL-1), IL-6 and tumor necrosis factor alpha (TNF-α), while increasing the anti-inflammatory cytokine IL-10, thereby helping to counteract the inflammation associated with certain malignancies. Finally, curcumin enhances immune-mediated tumor cell destruction by promoting the activity of natural killer (NK) cells and cytotoxic T lymphocytes (CTLs).[5–12]

In addition to its biophysical and photochemical properties, the chemical structure of curcumin also contributes significantly to its therapeutic potential. In particular, the aromatic ortho-methoxyphenol groups and the α,β-diketo moiety play important roles in mediating its biological activities and associated health benefits. Two naturally occurring curcumin analogs, demethoxycurcumin and bisdemethoxycurcumin, have been widely investigated for their pharmacological properties.

Despite its promising biological effects, curcumin has several limitations, including poor bioavailability, low stability, and limited aqueous solubility, which restrict its broader clinical application. Consequently, numerous

structural analogs and derivative compounds have been developed over time to overcome these drawbacks and enhance its therapeutic efficacy.[13,14]

Nanotechnology-based approaches provide strategies to prolong drug dissolution, enable targeted drug delivery and enhance transport across biological barriers. By carefully modifying the chemical composition, size and structural characteristics that regulate nanoparticle activity, a wide range of nanoformulations has been developed using organic, inorganic, lipid, and polypeptide-based materials, typically within the size range of 1–100 nm. These nanomaterials have been widely utilized as carriers for various anti-cancer drugs.[15–21]

The goal of this review was to consolidate the salient characteristics of curcumin and its cutting-edge nanoformulations, and to further outline the role of curcumin in the management of OSCC.

Material and methods

Study protocol

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for study selection, synthesis and reporting of results.[22] The study protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the registration number CRD42023483768.

Focused question

The focus of this literature review was to investigate the inhibitory effects of curcumin and its nanoformulations on cellular proliferation in OSCC at the molecular level. The review examined studies that explore the molecular mechanisms underlying the anti-cancer activity of curcumin and its nanoformulations. In addition, it analyzes the outcomes of in vitro and in vivo studies that have evaluated the effects of curcumin and its nanoformulations on the proliferation of OSCC cells.

Information sources and search strategy

The PubMed, Scopus and Cochrane Library databases were used to identify evidence-based research articles investigating the cytotoxic effects of curcumin and its nanoformulations on OSCC, published between January 2012 and August 2023. Following an initial study of the available literature, the following Medical Subject Headings (MeSH) phrase combination was used: ("Curcumin" OR "Curcuminoids" OR "Nanocurcumin" OR "Curcumin Analog" OR "Curcumin Microemulsions" OR "Desmethoxycurcumin" OR "Bisdemethoxycurcumin" OR "Curcumin Nanomicelles") AND ("Cisplatin-Resistant Human Oral Cancer Cells" OR "Cell Culture" OR "Cell Lines") AND ("Oral Squamous Cell Carcinoma" OR "Head and Neck Squamous Cell Carcinoma" OR "Oropharyngeal Carcinoma") AND ("MTA" OR "Flow Cytometry" OR "Annexin Assay" OR "Western Blot" OR "RT-PCR") AND ("Apoptosis" OR "Cytotoxicity" OR "Interleukin" OR "Signaling Pathway" OR "Photodynamic" OR "Epithelial–Mesenchymal Transition"). A manual check of the references for each of the included publications was executed to find studies that were not found in the online databases. The search was done by two reviewers independently, and screening was completed using the pre-established parameters.

Eligibility criteria

The eligibility criteria utilized in the present systematic review were established based on the PICOS criteria (Population, Intervention, Comparison, Outcome, and Study Design).

Population: Patients with OSCC aged 18 years and above.

Intervention: Free curcumin, curcuminoids or its nanoformulations.

Comparison: Placebo control group, or the administration of other synthetic chemotherapeutic drugs or radiotherapy.

Outcome: In vitro cellular proliferation, cytotoxicity or in vivo tumor dimensions, or tumor incidence, apoptosis, cell cycle arrest, and/or the evaluation of protein expression.

Study design: In vitro or clinical studies to evaluate the cytotoxicity effects of curcumin and its nanoformulations on OSCC.

Systematic reviews, questionnaire-based surveys, commentaries, case reports or case series, pilot studies, animal studies, and expert opinions were excluded. Articles not published in English or those that did not focus on the antitumor effects of curcuminoids or their nanoformulations were also excluded. The literature search, data extraction from the included studies, and quality assessment were independently performed by two expert reviewers. Any disagreement was resolved through discussion, and when necessary, a third reviewer was consulted to reach a consensus.

Study selection

The titles and abstracts of all the identified studies were independently screened by two reviewers. Publications that did not meet the eligibility criteria were excluded. The full texts of the selected articles were then independently evaluated by the same reviewers according to the predefined eligibility criteria. In addition, the reference lists of the included studies were manually searched to identify any further relevant publications. Any disagreement between the reviewers was resolved through discussion. If consensus could not be reached, a third reviewer was consulted to make the final decision. The final selec-

tion of studies was determined through agreement among all three reviewers.

Data extraction

The following information was extracted using a standardized data collection template: first author's name; study title; journal name; year of publication; country where the study was conducted; study design; assays and cell lines used; source of the cell lines; sample size and baseline characteristics; drug dosage; and key study findings. To minimize errors, data extraction was performed independently by two reviewers. When additional information was required, the corresponding author was contacted for clarification.

Quality assessment

The quality assessment of the included clinical studies was conducted using the Cochrane Collaboration Risk of Bias tool (RoB),[23] which categorizes the risk of bias as high, low, or unclear. The tool evaluates 7 domains: sequence generation; allocation concealment; blinding of participants and personnel; blinding of outcome assessment; incomplete outcome data; selective reporting; and other potential sources of bias. A low risk of bias was assigned when all criteria were fulfilled. A moderate risk of bias was designated when one domain was not adequately met. A high risk of bias was assigned when more than one domain was not satisfied.

The quality of the cell culture studies was evaluated using the World Cancer Research Fund International/University of Bristol (WCRF/UoB) framework,[24] which provides a scoring range from 0 to 6. A score of 0 was assigned to parameters that were not met or not reported. Studies were classified as low quality if they scored 0–2, moderate quality for scores of 3–4, and high quality for scores of 5–6. These scores reflected the overall study quality and were used to assess the degree of risk of bias for each cell culture study.

Results

Figure 1 illustrates the stepwise procedure for study selection. A total of 515 studies meeting the inclusion criteria were initially identified from the aforementioned databases, and an additional 47 articles were retrieved through manual search. After removing 114 duplicate records, 448 publications remained for title and abstract screening, resulting in the exclusion of 298 articles. Consequently, 150 full-text articles were assessed for eligibility. During full-text review, 115 studies were excluded due to deviations from the predefined criteria. Ultimately, 35 publications were included in the present systematic review, as depicted in the PRISMA flow chart.[25–59]

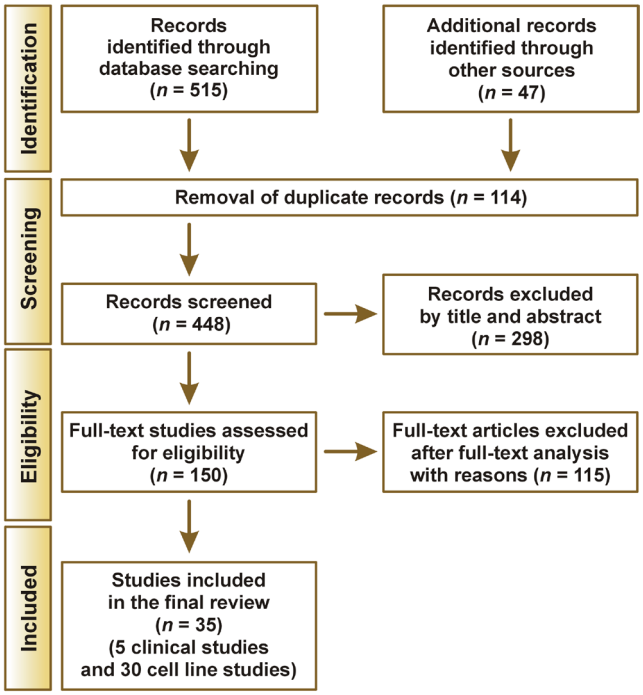

Fig. 1. PRISMA flow chart of the included studies (adapted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2009 flow diagram)

Five clinical studies[28,29,41,51,52] and 30 cell line investigations[25–27,30–40,42–50,53–59] were selected (Table 1, Table 2). Two of the 5 clinical trials were double-blind, randomized, placebo-controlled trials,[29,52] and one was single blinded.[28] The included studies employed a wide range of OSCC cell lines, with CAL-27 in 6 studies,[31,33,42,43,46,55] KB in 4 studies,[36,39,45,49] FaDu in 3 studies,[33,42,55] and SCC-9 in 3 studies[35,47,56] being the most common (Table 2).

General characteristics

The selected studies were carried out in several countries, including India,[28,34,39,48,49,53,54,59] China,[42,43,45,46,55,56,57,58] the USA,[29,35,41,52,55] South Korea,[25,36,37] Taiwan,[31,40,44] Brazil,[33,47] Germany,[38,51] Saudi Arabia,[26] Italy,[27] Spain,[30] Egypt,[32] and Japan.[50] All of the studies were published in English between 2012 and 2023. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, the Annexin assay for determining apoptosis, flow cytometry, the proliferation assay, Western blot, Northern blot, reactive oxygen species (ROS), real-time reverse transcriptase polymerase chain reaction (RT-PCR), the enzyme-linked immunosorbent assay (ELISA), and immune histochemistry (IHC) were employed in various studies. The curcumin doses utilized in the reviewed studies varied from 0 to 100 μm. Each of the entailed study found that when cell lines were treated with curcumin, there was an overall decrease in the proliferation of cells and cell growth when measured by the MTT assay, the luciferase assay and immunofluorescence. All of the in vitro studies had been conducted on human OSCC cell lines.

Table 1. Summary characteristics of the clinical studies included in the review

Study	Country	Study design	Sample size (*n*)	Age [years]	Gender M/F [%]	Intervention group and drug dosage	Control group	Assay	Study inference
Arun et al.[28] 2018	India	single-blinded RCT	I: 12 C: 9	NA	NA	cisplatin infusion (50 mg/m^2) weekly, along with radiotherapy and curcumin – 1.5 g/day (3 × 500 mg)	cisplatin infusion (50 mg/m^2) weekly, along with radiotherapy and placebo	CECT and the RECIST criteria	The curcumin group exhibited a marginal reduction in tumor dimension, with 58.3% showing a partial response and 41.7% showing stable disease; no statistically significant difference in comparison with the control group.
Basak et al.[29] 2020	USA	double-blind RCT	I: 12 C: 13	above 18	NA	transoral administration of APG-157 (3 × 100 mg and 3 × 200 mg)	healthy volunteers	multiplex immune-fluorescence, cytokine analysis and 16S rRNA gene sequencing	Curcumin caused a decrease in inflammatory markers and bacteroides species in the saliva, and an increase in immune T cells in tumor tissues.
Latimer et al.[41] 2015	USA	clinical trial	I: 15 C: 8	I: 52.7 ±11.5 C: 41.8 ±13.4	I: 93.3/6.7 C: 62.5/37.5	HNSCC; transmucosal administration of 4 g of microgranular curcumin	healthy volunteers	multiplex immunoassay and immune-histochemistry	A considerable decrease in the production of FGF-2, GM-CSF and IL-17 was documented.
Reiter et al.[51] 2012	Germany	ex vivo	mini organ culture of macroscopically healthy pharyngeal tissue of 40 patients with oropharyngeal SCC	NA	NA	tobacco smoke condensate (0.7 mg/dL) and 1 µM of curcumin	tobacco smoke condensate (0.7 mg/dL) and 0.1 µM of celecoxib	Comet assay, and Comet-FISH	DNA fragmentation was markedly decreased in the celecoxib and curcumin groups, with comparable anti-cancer potential.
Shin et al.[52] 2019	USA	double-blind, randomized, placebo-controlled phase I clinical trial	I: 12 C: 13	NA	NA	OSCC; oral administration of APG-157 (3 × 100 mg and 3 × 200 mg)	healthy volunteers	multiplex ELISA, 16S rRNA gene sequencing and immune-fluorescence	The drug had potent anti-tumor action without any adverse events; a marked increase in CD4 and CD8 infiltration was reported, and a dose-dependent reduction of inflammatory cytokines was noted.

M – male; F – female; RCT – randomized clinical trial; I – intervention group; C – control group; SSC – squamous cell carcinoma; HNSCC – head and neck squamous cell carcinoma; OSCC – oral squamous cell carcinoma; CECT – contrast-enhanced computed tomography; RECIST – Response Evaluation Criteria in Solid Tumors; FISH – fluorescence in situ hybridization; ELISA – enzyme-linked immunosorbent assay; FGF-2 – fibroblast growth factor-2; GM-CSF – granulocyte-macrophage colony-stimulating factor; IL-17 – interleukin 17; NA – data not available.

Table 2. Summary characteristics of the in vitro studies included in the review

Study	Country	Study design	Curcumin formulations	Cell lines	Source of cell lines	Culture conditions
Ahn et al.[25] 2012	South Korea	in vitro	curcumin and PDT	AMC-HN3	Asan Medical Center, South Korea	RPMI 1640 medium with 10% FBS, and 100 µg/mL streptomycin and 100 U/mL penicillin
Almalki et al.[26] 2021	Saudi Arabia	in vitro	combination of curcumin, desmethoxy-curcumin and bisdemethoxy-curcumin	HNO97	Cell Lines Services, USA	2% FBS-DMEM
Ardito et al.[27] 2018	Italy	in vitro	free curcumin	TSCC	NA	NA
Camacho-Alonso et al.[30] 2013	Spain	in vitro	curcumin, lycopene, and the combination of both	PE/CA-PJ15 cell lines irradiated	European Collection of Cell Cultures	DMEM with 10% FBS, 1% antibiotic
Chang et al.[31] 2013	Taiwan	in vitro	PLGA curcumin nanoparticles	CAL-27-cisplatin-resistant human OC cells	ATCC	DMEM with 10% FBS, 100 µg/mL streptomycin, 100 U/mL penicillin, 2 mM L-glutamine, and 100 µM cisplatin
Essawy et al.[32] 2022	Egypt	in vitro	nanocurcumin	SCC-4 and human gingival fibroblasts	ATCC and CERRMA	DMEM with 10% FBS, penicillin, streptomycin, trypsin/EDTA, and PBS
De Souza Ferreira et al.[33] 2019	Brazil	in vitro	P407/C974P/ curcumin	FaDu and CAL-27 (OSCC cells) and FNB6 (normal oral immortalized keratinocytes)	NA	RPMI for FaDu, DMEM for CAL-27, and Green's medium for FNB6
Ghosh et al.[34] 2018	India	in vitro	DNA curcumin complex with FdU nucleotides	UPCI-SCC-131	CSIR-IICB	DMEM with 10% FBS
Gosangari and Watkin[35] 2012	USA	in vitro	liposomes	SCC-9	ATCC	DMEM, F12 medium with 10% FBS
Jeon et al.[36] 2012	South Korea	in vitro	EF-24 and curcumin	KB cell line	ATCC	minimum essential medium containing non-essential amino acids with 5% FBS
Kim et al.[37] 2012	South Korea	in vitro	free curcumin	YD10B cell lines	Yonsei University, Seoul, South Korea	DMEM with 10% FBS, and 1% penicillin and streptomycin
Kötting et al.[38] 2021	Germany	in vitro and ex vivo	free curcumin	SCC-1 and SCC-4 cell lines; tumor specimens of 9 HNSCC patients	University of Düsseldorf, Germany	DMEM with 10% FBS
Kumbar et al.[39] 2022	India	in vitro	curcumin nanomicelles	cisplatin-resistant OC sublines Cis-KB	NA	DMEM with 10% FBS
Lai et al.[40] 2019	Taiwan	in vitro and in vivo	γ-PGA-gefitinib/ curcumin nanoparticles	SAS human OC cell line for in vitro and 20 four-week-old mice as the animal model	FIRDI, Taiwan	DMEM with 10% FBS, 2mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin
Liao et al.[42] 2018	China	in vitro and in vivo	free curcumin	CAL-27, FaDu cell lines and 4NQO mouse model	NA	DMEM with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin
C. Lin et al.[43] 2017	China	in vitro	curcumin analog diphenyl difluoroketone (EF24)	CAL-27 human OSCC cell line	HyClone; GE HealthCare Life Sciences, USA	DMEM with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin
H.Y. Lin et al.[44] 2012	Taiwan	in vitro	curcumin microemulsions	OSCC-4 and OSCC-25 cell lines	BCRC, Taiwan	DMEM and Ham's F12 medium
Lu et al.[45] 2012	China	in vitro	curcumin, F-CUR-L, CUR-L	KB, Hela and A549 cell lines	Chinese Academy of Science, Shanghai; Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China	RPMI 1640 folate-free medium with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin

Duration	Dosage	Assay	Study findings	Study inference
48 h	25 µM	MTT assay, morphological assay and Western blot	The PDT combined with curcumin increased cytotoxic and apoptotic action through the mitochondria-dependent apoptosis pathway and ROS generation.	These findings imply that the PDT plus curcumin combination method has a superior therapeutic effect in vitro.
48 h	35–65 µM	water-soluble tetrazolium salt, Annexin assay, Comet assay, and flow cytometry	Nanocurcumin significantly reduced the growth of HNO97 cells, and shows promise in preventing cancer development. Moreover, curcumin-induced DNA damage suggests an additional mechanism for the cytotoxic effects of curcuminoids.	Curcuminoids enhance apoptosis and inhibit the proliferation of tumor cells.
72 h	50 µM	MTT assay, apoptosis assay, and migration assay	The results suggest the potential utilization of curcumin as an anti-cancer agent in TSCC.	A marked decrease in cellular proliferation, as well as apoptosis were reported.
72 h	6.75 µM	MTT assay, apoptosis assay and ELISA	Cytotoxicity was increased with the curcumin and lycopene treatment. A synergetic effect was observed with the combination therapy.	The cytotoxic activity of curcumin and lycopene is increased in the PE/CA-PJ15 cell line.
24 h	40 µM	MTT assay, Western blot and RT-PCR	Curcumin nanoparticles enhanced apoptosis in CAR cells by modulating MRP1 and promoting ROS generation.	Curcumin nanoparticles cause CAL-27-cisplatin-resistant human OC cells to undergo apoptosis.
24 h	60.8 µg/mL	MTT assay, apoptosis assay and scratch wound healing assay	Nanocurcumin was found to possess a similar anti-tumorigenic effect as chemotherapeutic agents. Additionally, its remarkable luminescence makes it suitable to act as a dual theranostic agent, combining therapeutic and diagnostic capabilities.	Nanocurcumin has better biocompatibility and more potent cytotoxic effect than normal curcumin.
24 h	10–2,000 µM	MTT assay, anisotropy and rheology	The cytotoxicity testing showed that the formulations were selective in targeting cancer cells while sparing healthy cells.	The mucoadhesive nanostructured curcumin system enhances drug permeation and promotes selective activity against cancer cells.
48 h	40 µM	MTT assay and spectroscopy	Curcumin complexed with FdU nucleotides in a DNA hairpin structure exhibited enhanced cytotoxicity.	The complex is expected to be well tolerated, as curcumin and FdU-containing DNA exhibit low toxicity in vivo.
24 h	0.2, 0.1, 0.05, and 0.025 wt/wt	MTT assay and encapsulation efficiency assay	Increased bioavailabilty of the drug-loaded liposomes was observed with notable cytotoxic effects against tumor cells.	These findings suggest that various liposomal formulations can be used to meet specific in vivo requirements for cancer treatment.
24 h	10 µM of EF-24, 50 µM of curcumin	MTT assay, DNA fragmentation, Western blot, and caspase activity assay	EF-24 and curcumin fostered the proteolytic cleavage of caspases-3, -7 and -9, and increased apoptosis.	According to these findings, EF-24 and curcumin cause KB human OC cells to undergo apoptosis and stop proliferating.
24 h	40 mM	MTT assay and Western blot	Curcumin induces apoptosis and ROS generation in OSCC.	Curcumin inhibits OSCC with anti-cancer properties, through both autophagy and apoptosis.
48 h	20 µM	Western blot, ELISA, RT-qPCR, Annexin assay, and migration assay	The co-incubation of PIC with curcumin, but not with other NF-κB inhibitors, led to the inhibition of PIC-dependent NF-κB activation while enabling the unimpeded activation of the desired INF-I signaling pathway.	Curcumin inhibits the activation of NF-kB and has the potential for the reversal of the EMT process.
48 h	147.96 µg/mL	MTT assay, flow cytometry, dual staining, and DAPI assay	Curcumin nanomicelles induce apoptotic cell death by altering the mitochondrial membrane potential.	Controlled drug release resulted in increased cellular uptake and higher frequency of apoptotic cells (31%).
48 h	γ-PGA-Gef/Cur NPs with 20 µM of Gef and 10 µM of Cur	Western blot and flow cytometry	In the study, the animals were treated with PLGA NPs, free Gef/Cur, and γ-PGA-Gef/Cur NPs for 22 days. The measurements of animal body weight, tumor size and tumor weight were taken. The results indicated no significant difference in body weight. However, there were notable differences in tumor size between the free Gef/Cur and γ-PGA-Gef/Cur NPs groups.	The γ-PGA-Gef/Cur NPs considerably decreased tumor size as compared to the Gef/Cur group.
NA	NA	MTT assay and Western blot	Curcumin demonstrated an effective anti-tumor immune response by decreasing the number of regulatory T cells (Tregs) and MDSCs.	Both the experiments revealed PD-L1 and p-STAT3^{Y705} inhibition, indicating an anti-tumor response.
24 h	30 µM	MTT assay and Western blot	The present findings showed that EF24 increased the amount of cytoplasmic cytochrome c in CAL-27 cells, which lowered cell viability.	EF24 has a higher cytotoxic effect on CAL-27 cells than cisplatin.
48 h	15 µM	MTT assay and zeta potential assay	Curcumin microemulsions revealed an enhanced cytotoxic effect when combined with ultrasound administration, notably on OSCC-25 cells.	The microscopic examination of the cells revealed cell rupture and damage, following treatment, confirming the harmful effects of curcumin microemulsions.
24 h	14.5 µg/mL for free curcumin, 16.3 µg/mL for F-CUR-L and 29.5 µg/mL for CUR-L	MTT assay, Annexin and flow cytometry	F-CUR-L increased curcumin solubility and specifically targeted the folate receptor- positive tumor cell line.	Both dose- and time-dependent apoptosis was observed in the 2 types of tumor cells treated with F-CUR-L.

Table 2 – continuation

Study	Country	Study design	Curcumin formulations	Cell lines	Source of cell lines	Culture conditions
Ma et al.[46] 2020	China	in vitro	free curcumin	CAL-27 tongue cancer cell line	Procell Life Science & Technology, China	DMEM with 1% penicillin/ streptomycin and 20% FBS
Mazzarino et al.[47] 2015	Brazil	in vitro	curcumin-loaded, chitosan-coated polycaprolactone nanoparticles	SCC-9 human OSCC	Rio de Janeiro, Brazil	DMEM, F12 with 10% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, 10 mM HEPES, and 400 ng/mL hydrocortisone
Mishra et al.[48] 2015	India	in vitro	free curcumin	93VU147T cell lines	VU Medical Center, Molecular Pathology Unit, Amsterdam, the Netherlands	DMEM with 10% FBS
Mukherjee et al.[49] 2022	India	in vitro	nanocurcumin, cetuximab, and the combination of both	KB-3-1 cell line	NCCS	DMEM with 10% FBS, 1% antibiotic-antimycotic solution
Ohnishi et al.[50] 2020	Japan	in vitro	free curcumin	HSC-4 and Ca9-22	RIKEN BioResource Research Center, Japan	DMEM with 10% FBS
Singh et al.[53] 2014	India	in vitro	nanoformulated curcumin–silica nanocomplex	SCC-4451 cell line	Institute of Nuclear Medicine and Allied Sciences, Delhi, India	DMEM with 10% FBS
Sivanantham et al.[54] 2016	India	in vitro	curcumin and/ or 5-fluorouracil, doxorubicin or cisplatin	NT8e	Advanced Centre for Training, Research and Education on Cancer, Tata Memorial Hospital, India	DMEM with 10% FBS
Xi et al.[55] 2015	China and USA	in vitro	curcumin	FaDu and CAL-27	ATCC	DMEM with 10% FBS
Xiao et al.[56] 2014	China	in vitro	free curcumin	SCC-9	ATCC	NA
Zhen et al.[57] 2014	China	in vitro	free curcumin	SCC-25	ATCC	DMEM with 10% FBS, 100 U/ mL penicillin, 100 µg/mL streptomycin
Liu et al.[58] 2021	China	in vitro	free curcumin	HSC3 and CAL-33 cell lines	University of Wuhan; Hubei University of Medicine, Shiyan, China	DMEM with 10% FBS, 1% antibiotic
Srivastava et al.[59] 2018	India	in vitro	5-fluorouracil and curcumin nanoemulsions	SCC-090 and SCC-152 cell lines	NCCS	DMEM with 10–12% FBS, 1% streptomycin and penicillin cocktail

PDT – photodynamic therapy; PLGA – poly(lactic-co-glycolic acid); FdU – 5-fluoro-2′-deoxyuridine; γ-PGA – γ-polyglutamic acid; F-CUR-L – folate-receptor-targeted liposomal curcumin; CUR-L – non-targeted liposomal curcumin; OC – oral cancer; ATCC – American Type Culture Collection; CERRMA – Center of Excellence for Research in Regenerative Medicine and Application; CSIR-IICB – Indian Institute of Chemical Biology; FIRDI – Food Industry Research and Development Institute; BCRC – Bioresource Collection and Research Center; NCCS – National Centre for Cell Science; RPMI 1640 medium – Roswell Park Memorial Institute 1640 medium; FBS – fetal bovine serum; DMEM – Dulbecco's modified Eagle medium; EDTA – ethylenediaminetetraacetic acid; PBS – phosphate-buffered saline; HEPES – 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RT-PCR – real-time reverse transcriptase polymerase chain reaction; DAPI – 4′,6-diamidino-2-phenylindole; CCK-8 – Cell Counting Kit-8; SRB – sulforhodamine B; ROS – reactive oxygen species; TSCC – tongue squamous cell carcinoma; MRP1 – multidrug resistance protein 1; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; PIC – polyinosinic-polycytidylic acid; INF-1 – type-I interferon; EMT – epithelial-mesenchymal transition; MDSC – myeloid-derived suppressor cell; PD-L1 – programmed death-ligand 1; p-STAT3^{Y705} – phospho-signal transducer and activator of transcription 3; AP-1 – activator protein 1; HGF – hepatocyte growth factor; ERK – extracellular signal-regulated kinase; Bax – Bcl-2-associated X protein; PARP – poly(ADP-ribose) polymerase; Bcl-2 – B-cell lymphoma 2; EGFR –epidermal growth factor receptor; Akt – protein kinase B; ERK1/2 – extracellular signal-regulated kinases 1 and 2; Sp1 – specificity protein 1; p65 – transcription factor p65; HSF1 – heat shock factor 1; NA – data not available.

Duration	Dosage	Assay	Study findings	Study inference
24 h	100 µM	CCK-8, TUNEL assay, Western blot, and scratch wound healing assay	This study uncovered the anti-proliferative and pro-apoptotic effects of curcumin in CAL-27 cells.	Curcumin promotes apoptosis through the modulation of oxygen-related signaling pathways and exhibits potential anti-cancer effects.
72 h	500 µg/mL	MTT assay and zeta potential assay	Following treatment with curcumin-loaded, chitosan-coated polycaprolactone nanoparticles, drug retention in the mucosa indicates that these systems may be useful for the local treatment of a variety of illnesses. Furthermore, in vitro experiments showed that the nanoparticles dramatically reduced the survival of SCC-9 human OC cells by triggering apoptosis.	The mucoadhesive curcumin nanoparticles coated with chitosan showed increased cytotoxicity in the treatment of OC through oral administration.
72 h	100 µM	MTT assay, Northern blot and PCR	Curcumin inhibits transcription factors, such as AP-1 and NF-kB, and selectively inhibits transcription of *HPV16/E6* oncogene in OC cell lines.	Curcumin causes *HPV16*-infected OC cells (93VU147T) to undergo apoptosis.
24 h	335.1 µg/mL	MTT assay	The cytotoxic effect of nanocurcumin on OSCC cells was found to be dependent on its concentration. Additionally, when combined with nanocurcumin, cetuximab induced greater cell death as compared to cetuximab alone. These results suggest the potential chemo-adjuvant effect of nanocurcumin.	A significant apoptosis was observed in the nanocurcumin group as compared to the cetuximab group. The combined therapy was more effective than a single-drug regimen.
48 h	15 µM	Western blot, migration assay and gelatin zymography	It was shown that curcumin could inhibit HGF-induced EMT and cell motility in the OSCC cell line by blocking c-Met and inhibiting the ERK effector pathway.	The findings suggest that curcumin is a powerful drug that targets invasive OC cells.
24 h	25 µM	MTT assay and Western blot	Curcumin–SiNPs revealed enhanced bioavailability, and hence higher cytotoxic effects against OC cells than free curcumin due to the inhibition of NF-κB action.	The enhanced photodynamic activity of curcumin delivered via SiNPs in OC cells, together with the rapid accumulation of the nanoformulated drug, suggests that SiNPs may serve as a promising delivery vehicle.
48 h	15 µM	SRB assay, nuclear morphology with DAPI, flow cytometry, and Western blot	Curcumin combined with 5-fluorouracil or doxorubicin treatment caused apoptosis in NT8e cells by raising Bax, caspase-3 and PARP, and by inhibiting Bcl-2. This was further supported by y DAPI staining, which revealed typical apoptotic features in cells, including membrane blebbing, nuclear condensation and cell shrinkage.	Curcumin, along with doxorubicin or 5- fluorouracil, showed increased cytotoxicity, cell cycle arrest and apoptosis.
24 h	12.5 µM	CCK-8, DNA fragmentation and Western blot	The findings revealed that curcumin inhibited pro-survival signaling pathways, leading to apoptosis and the suppression of HNSCC FaDu cell growth through the activation of the pro-apoptotic protein Bik.	Curcumin inhibits cell growth in a dose- and time-dependent manner, and increases apoptosis by activating the intrinsic mitochondria-mediated pathway.
72 h	60 µM	MTT assay, Western blot and RT-PCR	Current findings imply that curcumin can suppress the growth of SCC-9 cells by promoting the production of miR-9 and interfering with the Wnt/β-catenin signaling pathway.	The results showed that curcumin upregulated the expression of miR-9, which decreased the growth of OSCC cells.
48 h	40 µM	MTT assay, Western blot and RT-PCR	Curcumin inhibits cellular proliferation through the inhibition of the phosphorylation of EGFR and EGFR downstream signaling of Akt, ERK1/2 and STAT3.	Through the EGFR signaling pathways, curcumin mediates the proliferation and invasion of OSCC cells.
48 h	20 µM	Western blot, RT-qPCR, Dual-Luciferase Reporter, and CCK-8	The study showed that curcumin inhibited the proliferation and NF-κB activity of OSCC cells.	Curcumin decreases the production of Sp1, p65, and HSF1, and inhibits NF-κB activation in OC cells.
96 h	320 µg/mL	MTT assay and Western blot	The substantial induction of apoptosis in OSCC cells, SCC-090 and SCC-152, was demonstrated by alterations in protein expression, particularly in Blc2, Bax, p53, and p21.	5-fluorouracil and curcumin nanoemulsions exhibited enhanced apoptotic effects via multimodal therapeutic mechanisms against OSCC cells.

Role of curcumin in OSCC management

Curcumin has been shown to inhibit regulatory T cell (Treg) migration and induce S-phase cell cycle arrest. It has also been reported to reverse epithelial-mesenchymal transition (EMT), a process associated with tumor invasion and metastasis. Mishra et al. demonstrated that curcumin reduced cell viability and induced morphological changes in human papillomavirus (HPV)-positive oral cancer (OC) cell lines.[48] Northern blot analysis indicated that curcumin suppressed the E6-oncogene-mediated degradation of p53 in these HPV-positive cancer cells. At a concentration of 100 μM, curcumin inhibited the expression of E6, NF-κB and activator protein 1 (AP-1) in HPV-positive cell lines.[48] Additionally, Xiao et al. reported that curcumin enhanced the expression of miR-9, which reduces the proliferation of SCC-9 cells and suppresses the Wnt/β-catenin signaling pathway.[56]

Zhen et al. conducted an in vitro study showing that curcumin reduced the proliferation of SCC-25 cells and induced G2/M phase cell cycle arrest in a dose-dependent manner.[57] The study also demonstrated that curcumin suppressed the expression of MMP-2 and MMP-9, as well as urokinase-type plasminogen activator (uPA) and its receptor (uPAR). Curcumin was found to modulate phosphorylated epidermal growth factor receptor (p-EGFR) and downstream signaling mediators, including protein kinase B (Akt), extracellular signal-regulated kinases 1 and 2 (ERK1/2) and STAT3. Additionally, curcumin inhibited EGF-induced EGFR phosphorylation and reduced EGF-stimulated SCC-25 cell invasion.[57]

Both Almalki et al.[26] and Zhen et al.[57] reported G2/M phase arrest in curcuminoid-treated cells after 6 h of treatment and demonstrated the ability of curcumin to induce DNA damage in these cells. According to Lu et al., curcumin exhibited improved solubility and stability when encapsulated in folate-linked liposomes.[45] Chang et al. showed that curcumin-loaded nanoparticles induced apoptosis and significantly inhibited proliferation in OSCC cell lines while demonstrating lower cytotoxicity in normal cells, highlighting the specificity of nano-based drug delivery.[31] Conversely, Mazzarino et al. observed that free curcumin (IC_{50} (half-maximal inhibitory concentration) = 93.4 μM) was more cytotoxic than curcumin nanoformulations (IC_{50} = 271.5 μM) after 24 h of treatment.[47]

Role of nanocurcumins in OSCC management

Several innovative strategies have been developed to design curcumin-based nanomaterials as targeted drug-delivery systems. Polymer nanoparticles are particularly effective due to their excellent biocompatibility and prolonged circulation in the bloodstream. Srivastava et al. reported that curcumin nanoparticles exhibited chemoprotective effects in OC cells, mitigating the adverse reactions typically induced by 5-fluorouracil.[59] Nanocurcumin also demonstrated antioxidant properties and modulated the expression of apoptotic proteins, including Bcl-2 and Bcl-2-associated X protein (Bax), in the treated cells.[59]

Another study investigated the anti-tumor efficacy of gefitinib and curcumin-loaded nanoparticles in human OC SAS cells in vitro and in SAS xenograft tumors in vivo.[40] The results showed that γ-polyglutamic acid (γ-PGA)-coated gefitinib/curcumin nanoparticles were effectively internalized by SAS cells, significantly reducing viable cell counts. Both free Gefitinib/curcumin and γ-PGA-gefitinib/nanocurcumin induced apoptotic cell death via caspase-3, caspase-9 and mitochondria-mediated pathways. In vivo experiments further demonstrated that γ-PGA-gefitinib/nanocurcumin significantly inhibited tumor growth as compared to the untreated or free gefitinib/curcumin-treated groups.[40]

Essawy et al. investigated the effects of curcumin nanoparticles on OC cells, using a simple and cost-effective solvent–antisolvent precipitation method.[32] Unlike doxorubicin, which primarily induced necrosis in these cell lines, curcumin nanoparticles triggered cytotoxicity predominantly through apoptosis. The study also highlighted the intrinsic luminescence of curcumin nanoparticles, suggesting their potential as dual-function theranostic agents.[32]

Another study reported that the combined treatment with 200-nm nanocurcumin and cetuximab enhanced cytotoxicity as compared to cetuximab monotherapy, indicating the potential chemo-adjuvant role of curcumin nanomaterials in OC therapy.[49]

De Souza Ferreira et al. developed nanostructured gel formulations, incorporating curcumin for OC treatment.[33] These formulations facilitated the rapid absorption and localization of curcumin in the hydrophobic core of nano-sized polymeric micelles, resulting in prolonged retention in the oral cavity.[39] Cytotoxicity assays revealed that these nanoformulations selectively targeted tumor cells while sparing normal cells.[33] Such strategies improve the physicochemical properties of curcumin by enhancing its solubility and tissue penetration, thereby augmenting its anti-cancer activity. Given these promising findings, further preclinical and clinical studies are warranted to evaluate the therapeutic potential of novel curcumin formulations.

Lin et al. conducted a study to evaluate the effects of curcumin microemulsions on OC cell lines.[44] The results demonstrated that short-term treatment with curcumin in microemulsion form exerted significant cytotoxic effects on cancer cells. In SCC-25 cells, these effects were further enhanced when ultrasound was applied. This enhancement was likely due to the improved cellular delivery of curcumin, facilitated by the interaction of microemulsion particles with cell membranes or

by overcoming transport barriers through ultrasound-induced membrane permeabilization and localized heating.[44] When combined with ultrasound, these biodegradable microemulsions may provide controlled drug release and improved tissue-targeting capabilities. Additionally, curcumin nanoparticles have been shown to possess significantly higher bioavailability and improved aqueous solubility as compared to conventional curcumin, which may enhance their therapeutic potential.

Chang et al. demonstrated that water-soluble poly(lactic-co-glycolic acid) (PLGA)-based nanocurcumin enhanced therapeutic efficacy in CAL-27 cisplatin-resistant (CAR) OC cells.[31] The nanocurcumin formulation promoted the generation of ROS and increased the expression of apoptosis-related molecules, including cleaved caspase-3/caspase-9, apoptotic protease activating factor 1 (Apaf-1), cytochrome c, and Bax, while reducing the expression of the anti-apoptotic protein Bcl-2. These effects collectively activated the intrinsic apoptotic pathway in CAR cells.[31] Additionally, nanocurcumin modulates the structure and activity of multidrug-resistance proteins while enhancing ROS production, thereby contributing to increased cancer cell susceptibility to treatment.[53] Organically modified silica nanomaterials used as carriers for curcumin delivery in OC cells demonstrated improved cellular uptake of curcumin and enhanced phototoxic effects in cancer cells, highlighting the potential of silica-based nanocarriers to improve the anti-cancer activity of curcumin.[25,53]

Mazzarino et al. evaluated the therapeutic potential of mucoadhesive polycaprolactone curcumin nanostructures coated with chitosan for the treatment of OC.[47] Ghosh et al. developed a multifunctional nanoconjugate by integrating the surface of gold nanorods (GNRs) with a cytotoxic nucleoside, 5-fluoro-2'-deoxyuridine (FdU), incorporated into a DNA hairpin structure along with a hydrophobic curcumin complex.[34] The study demonstrated that curcumin could be successfully incorporated into small DNA hairpins to improve drug delivery efficiency. When combined with FdU nucleotides, this system significantly enhanced cytotoxicity in SCC-131 cells, suggesting its potential application as a therapeutic strategy for advanced-stage cancers.[34]

Clinical trials on curcumin in OSCC

The present review reported elevated levels of cytokines in the salivary supernatant of cancer patients, with reduced concentrations observed following treatment with APG-157, particularly for the pro-inflammatory cytokines IL-1 and IL-8. These findings suggest that APG-157 may suppress tumor-associated inflammation by reducing levels of NF-κB–regulated cytokines that contribute to inflammatory responses.[29]

Reiter et al. also demonstrated significant DNA damage caused by smoke condensate, primarily as a result of oxidative stress.[51] In this context, curcumin and celecoxib were shown to provide protective effects against such DNA damage.[51]

In the study conducted by Arun et al., 21 patients with OSCC were randomly allocated into 2 groups – group A, comprising 12 patients, and group B, comprising 9 patients.[28] The baseline tumor dimensions were assessed using contrast-enhanced computed tomography (CECT). After 3 months of treatment, patients were re-evaluated using CECT scans, and treatment responses were assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST), which categorize outcomes as complete response, partial response, progressive disease, or stable disease.[28]

Latimer et al. reported a significant increase in the serum levels of microgranular curcumin administered via the transmucosal route within 30 min of ingestion in both cancer patients and healthy volunteers.[41]

Quality assessment of the studies reviewed

When evaluated using the RoB, all the included clinical trials were considered to have a high risk of bias (Fig. 2). This classification was primarily due to the incomplete reporting of the key methodological aspects, particularly allocation concealment and blinding procedures, which are essential components of rigorous quality assessment.

Using the WCRF/UoB framework for evaluating cell line studies, 25 studies were rated as high quality, achieving scores between 5 and 6, while 5 studies were categorized as moderate quality with scores ranging from 3 to 4 (Table 3).

Fig. 2. Risk of bias of the reviewed clinical studies (Cochrane Collaboration Risk of Bias tool (RoB))

Table 3. Quality assessment of the cell line studies included in the review, based on the WCRF International/UoB recommendations

Study	Source	Culture medium	Replicates	Controls	Multiple cell lines	Selective reporting	Total
Ahn et al.[25] 2012	1	1	1	1	0	1	5
Almalki et al.[26] 2021	1	1	1	1	1	1	6
Ardito et al.[27] 2018	0	1	1	1	0	1	4
Camacho-Alonso et al.[30] 2013	1	1	1	1	0	1	5
Chang et al.[31] 2013	1	1	1	1	0	1	5
Essawy et al.[32] 2022	1	1	1	1	1	1	6
De Souza Ferreira et al.[33] 2019	0	1	1	0	1	1	4
Ghosh et al.[34] 2018	1	1	1	1	0	1	5
Gosangari and Watkin[35] 2012	1	1	1	1	0	1	5
Jeon et al.[36] 2012	1	1	1	1	0	1	5
Kim et al.[37] 2012	1	1	1	1	0	1	5
Kötting et al.[38] 2021	1	1	1	1	1	1	6
Kumbar et al.[39] 2022	0	1	1	1	0	1	4
Lai et al.[40] 2019	1	1	1	1	0	1	5
Liao et al.[42] 2018	0	1	1	1	1	1	5
C. Lin et al.[43] 2017	1	1	1	1	0	1	5
H.Y. Lin et al.[44] 2012	1	1	1	1	1	1	6
Lu et al.[45] 2012	1	1	1	1	1	1	6
Ma et al.[46] 2020	1	1	0	0	0	1	3
Mazzarino et al.[47] 2015	1	1	1	1	0	1	5
Mishra et al.[48] 2015	1	1	1	1	0	1	5
Mukherjee et al.[49] 2022	1	1	1	1	0	1	5
Ohnishi et al.[50] 2020	1	1	1	1	1	1	6
Singh et al.[53] 2014	1	1	1	0	0	1	4
Sivanantham et al.[54] 2016	1	1	1	1	0	1	5
Xi et al.[55] 2015	1	1	1	1	1	1	6
Xiao et al.[56] 2014	1	1	1	1	0	1	5
Zhen et al.[57] 2014	1	1	1	1	1	1	6
Liu et al.[58] 2021	1	1	1	1	1	1	6
Srivastava et al.[59] 2018	1	1	1	1	1	1	6

Discussion

Curcumin exhibits antimicrobial, antifungal, antiviral, anti-inflammatory, and pro-apoptotic properties. Despite significant advancements in chemotherapy and an improved understanding of the molecular pathophysiology of OC, the survival rates have remained relatively low, ranging from approx. 50% to 59% in recent years.[60] Curcumin has been extensively investigated in numerous in vivo and in vitro models of OSCC worldwide. Studies have reported that curcumin reduces the proliferation of OSCC cell lines in a dose-dependent manner in both in vitro and in vivo settings.[61]

Curcumin modulates multiple signaling pathways in cancer cells, reduces the expression of the proteins associated with therapeutic resistance and enhances the efficacy of anti-cancer drugs at various stages of treatment. It may also inhibit drug resistance mechanisms by increasing the sensitivity of chemotherapy-resistant cells.[62] Nanotechnology offers promising opportunities for developing medications with nanoscale dimensions, and nanocarrier-based drug delivery systems are being explored to improve the physicochemical properties and therapeutic efficacy of the drugs administered to these patients.[60]

Curcumin has been shown to suppress cellular proliferation and tumor metastasis while promoting apoptosis in OC cell lines through the modulation of multiple signaling pathways.[7] The synergistic effects of curcumin in combination with the established chemotherapeutic agents, radiotherapy, the emerging therapeutic strategies, or other plant-derived compounds have been widely investigated across different tumor types. These studies have reported enhanced therapeutic benefits as compared to the conventional kinds of treatment alone.

Similar findings have been obtained in OSCC cell lines and experimental animal models when curcumin was combined with epigallocatechin gallate (EGCG), resveratrol and photodynamic therapy (PDT). Moreover, the interaction of curcumin with radiotherapy or chemotherapeutic agents such as cisplatin and 5-fluorouracil – commonly used in OSCC treatment – has been shown to significantly enhance anti-cancer efficacy. These outcomes are consistent with the conclusions of a previous systematic review.[63]

The development of innovative photodynamic nanotechnologies incorporating precise anti-cancer nanotheranostic systems has attracted considerable interest over recent decades. A novel strategy has been developed using tumor microenvironment–responsive, near-infrared–sensitive GNR drug conjugates to enable integrated plasmonic photothermal therapy for cancer treatment.[64]

Multiple preclinical studies have demonstrated the anti-cancer potential of curcumin in various cancers. Curcumin has been shown to induce G2/M phase cell cycle arrest, trigger apoptosis and autophagy, and disrupt the key molecular signaling pathways involved in tumor progression. It also interferes with tumor cell invasion and metastasis, thereby enhancing the therapeutic efficacy of currently used chemotherapeutic agents. Furthermore, the suppression of pro-inflammatory cytokines by curcumin may significantly reduce the inflammation associated with cancer progression. Curcumin also participates in additional signaling mechanisms, including the induction of neutrophil activation and degranulation through increased cell surface expression of markers such as CD35, CD66b and CD63.[65]

Several strategies related to drug formulation and delivery systems have been developed to overcome the limited oral absorption of curcumin caused by its poor solubility. In this review, we analyzed studies that investigated free curcumin, curcumin in combination with other agents, curcumin nanoformulations, and curcumin analogs. Among the included studies, 11 investigations evaluated free curcumin (37%), 10 examined curcumin nanoformulations (33%), 7 explored curcumin in combination with other agents (23%), and 2 investigated curcumin analogs (7%). The findings indicate that curcumin exhibits biological activity even in its unmodified form.

Although numerous novel curcumin nanoformulations have demonstrated promising antineoplastic, theranostic and chemo-adjuvant properties, the field of nanotechnology remains relatively new and still faces several limitations. One of the major technical challenges in drug development is controlling the size of curcumin nanoparticles for effective drug delivery applications. In addition, these technologies are often expensive and have not yet been widely implemented. Nevertheless, nanoparticle-based delivery systems have the potential to overcome the limitations of the low bioavailability of curcumin by improving its stability, enhancing cellular uptake and enabling controlled drug release. Therefore, such approaches may offer greater therapeutic promise than the use of free curcumin alone.[66]

Extensive research has been conducted to develop improved curcumin formulations. Various delivery systems, including liposomes, nanoemulsions, micelles and nanostructured lipid carriers, have been explored to enhance curcumin bioavailability. However, the surfactants and organic solvents used in the preparation of these formulations may introduce potential toxicological concerns, which should be carefully considered in future development.[67]

The U.S. Food and Drug Administration (FDA) (2013) classified curcumin as generally safe, with no significant adverse effects reported. Some isolated cases of transient allergic dermatitis have been documented.[68] Dose-escalation studies have indicated that daily consumption of curcumin at doses up to 12 g does not produce serious toxic effects.[69] Furthermore, the clinical investigations involving patients with solid tumors reported no significant adverse events following the oral administration of curcumin at a dose of 900 mg/day for 8 weeks, apart from mild gastrointestinal discomfort.[70]

A major limitation of the present review is that a meta-analysis could not be performed due to the heterogeneity of the available data.

Recommendations for the future

Although nanotechnology-based formulations have shown promise in overcoming the limited bioavailability of curcumin, these systems often lack adequate cellular specificity. While many innovative curcumin nanoformulations demonstrate notable anti-tumor and chemo-adjuvant properties, several technological challenges remain in their development. One of the primary challenges is the precise control of nanoparticle size to achieve effective targeted drug delivery. In addition, these technologies are costly and have not yet been widely commercialized.

Polymeric nanoparticles and liposomal systems have been shown to improve curcumin bioavailability. However, the potential risk of off-target toxicities has not been sufficiently investigated. Curcumin has been reported to exert cytotoxic effects on cancer cells while displaying cytoprotective effects on normal cells at different dosages and concentrations. These observations should be carefully considered in future preclinical and clinical studies evaluating novel curcumin formulations.

Furthermore, heterogeneity in study designs, formulation types, sample sizes, and routes of administration in human cancer studies makes it difficult to determine which formulation possesses the most favorable pharmaceutical characteristics.[66] Many of these promising formulations have yet to undergo extensive clinical trials and require the development of cost-effective manufacturing methods. Therefore, further comprehensive in vivo studies and well-designed clinical trials are necessary to establish their potential therapeutic applications.

Conclusions

The combination therapies incorporating nanoformulations have gained considerable attention as promising drug delivery strategies that may overcome several limitations of the conventional treatment. These systems can enhance intracellular drug concentrations and improve synergistic therapeutic efficacy in cancer management. Novel curcumin formulations have been explored for their potential role in the treatment of OC, with encouraging outcomes.

Curcumin has been shown to exert beneficial effects on OSCC cell lines by reducing cell growth and viability, primarily through the induction of apoptosis via the intrinsic pathway and by causing cell cycle arrest at the G2/M phase. Furthermore, clinical studies have indicated that curcumin may reduce tumor size and exhibit anti-inflammatory and cytotoxic effects at the cellular level.

However, further research is needed to better understand how advanced delivery systems, such as liposomes and polymeric nanoparticles, influence the therapeutic efficacy of curcumin. In addition, comprehensive investigations into the bio-interactions between curcumin nanoparticles and cellular components are required to clarify the mechanisms responsible for their selective cellular uptake and activity.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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During the preparation of this manuscript, the authors used DeepL Translate for language checking/grammar correction. After its use, the authors thoroughly reviewed, verified and revised all content to ensure accuracy and originality. The authors take full responsibility for the integrity and final content of the published article.

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References

1. Wilken R, Veena MS, Wang MB, Srivatsan ES. Curcumin: A review of anti-cancer properties and therapeutic activity in head and neck squamous cell carcinoma. *Mol Cancer*. 2011;10:12. doi:10.1186/1476-4598-10-12
2. Kabir MT, Rahman MH, Akter R, et al. Potential role of curcumin and its nanoformulations to treat various types of cancers. *Biomolecules*. 2021;11(3):392. doi:10.3390/biom11030392
3. Utaipan T, Boonyanuphong P, Chuprajob T, Suksamrarn A, Chunglok W. A trienone analog of curcumin, 1,7-bis(3-hydroxyphenyl)-1,4,6-heptatrien-3-one, possesses ROS- and caspase-mediated apoptosis in human oral squamous cell carcinoma cells in vitro. *Appl Biol Chem*. 2020;63:7. doi:10.1186/s13765-020-0491-8
4. Zoi V, Galani V, Lianos GD, Voulgaris S, Kyritsis AP, Alexiou GA. The role of curcumin in cancer treatment. *Biomedicines*. 2021;9(9):1086. doi:10.3390/biomedicines9091086
5. Davoodvandi A, Farshadi M, Zare N, et al. Antimetastatic effects of curcumin in oral and gastrointestinal cancers. *Front Pharmacol*. 2021;12:668567. doi:10.3389/fphar.2021.668567

6. Doello K, Ortiz R, Alvarez PJ, Melguizo C, Cabeza L, Prados J. Latest in vitro and in vivo assay, clinical trials and patents in cancer treatment using curcumin: A literature review. *Nutr Cancer*. 2018;70(4):569–578. doi:10.1080/01635581.2018.1464347
7. Singh AK, Sharma NK, Mishra N, et al. Effects of curcumin on oral cancer at molecular level : A systematic review. *Natl J Maxillofac Surg*. 2023;14(1):9–15. doi:10.4103/njms.njms_29_22
8. Erdilek D, Gümüştaş B, Güray Efes B. Digitalization era of dental education: A systematic review. *Dent Med Probl*. 2023;60(3):513–525. doi:10.17219/dmp/156804
9. Alshiddi IF. Survival rate and clinico-radiographic parameters around narrow-diameter dental implants for fixed dental prostheses in the posterior regions: A systematic review. *Dent Med Probl*. 2023;60(2):345–353. doi:10.17219/dmp/140757
10. Dommeti VK, Pramanik S, Roy S. Design of customized coated dental implants using finite element analysis. *Dent Med Probl*. 2023;60(3):385–392. doi:10.17219/dmp/142447
11. Viswanath Reddy LK, Madithati P, Narapureddy BR, et al. Perception about health applications (apps) in smartphones towards telemedicine during COVID-19: A cross-sectional study. *J Pers Med*. 2022;12(11):1920. doi:10.3390/jpm12111920
12. Rathi S, Chaturvedi S, Abdullah S, et al. Clinical trial to assess physiology and activity of masticatory muscles of complete denture wearer following vitamin D intervention. *Medicina (Kaunas)*. 2023;59(2):410. doi:10.3390/medicina59020410
13. Chuprajob T, Changtam C, Chokchaisiri R, Chunglok W, Sornkaew N, Suksamrarn A. Synthesis, cytotoxicity against human oral cancer KB cells and structure–activity relationship studies of trienone analogues of curcuminoids. *Bioorg Medl Chem Lett*. 2014;24(13):2839–2844. doi:10.1016/j.bmcl.2014.04.105
14. Piwowarczyk L, Stawny M, Piwowarczyk K, et al. Role of curcumin in selected head and neck lesions. Limitations on the use of the Hep-2 cell line: A critical review. *Biomed Pharmacother*. 2022;154:113560. doi:10.1016/j.biopha.2022.113560
15. Navya PN, Kaphle A, Srinivas SP, Bhargava SK, Rotello VM, Daima HK. Current trends and challenges in cancer management and therapy using designer nanomaterials. *Nano Converg*. 2019;6(1):23. doi:10.1186/s40580-019-0193-2
16. Ronsivalle V, Carli E, Lo Giudice A, Lagravère M, Leonardi R, Venezia P. Nasal septum changes in adolescents treated with tooth-borne and bone-borne rapid maxillary expansion: A CBCT retrospective study using skeletal tortuosity ratio and deviation analysis. *Children (Basel)*. 2022;9(12):1853. doi:10.3390/children9121853
17. Lo Giudice A, Ronsivalle V, Santonocito S, et al. Digital analysis of the occlusal changes and palatal morphology using elastodontic devices. A prospective clinical study including Class II subjects in mixed dentition. *Eur J Paediatr Dent*. 2022;23(4):275–280. doi:10.23804/ejpd.2022.23.04.04
18. Fama F, Cicciu M, Sindoni A, et al. Maxillofacial and concomitant serious injuries: An eight-year single center experience. *Chin J Traumatol*. 2017;20(1):4–8. doi:10.1016/j.cjtee.2016.11.003
19. Cervino G, Fiorillo L, Spagnuolo G, et al. Interface between MTA and dental bonding agents: Scanning electron microscope evaluation. *J Int Soc Prev Community Dent*. 2017;7(1):64–68. doi:10.4103/jispcd.JISPCD_521_16
20. Bollero P, Franco R, Cecchetti F, et al. Oral health and implant therapy in Parkinson's patients: Review. *Oral Implantol (Rome)*. 2017;10(2):105–111. doi:10.11138/orl/2017.10.2.105
21. Franco R, Gianfreda F, Miranda M, Barlattani A, Bollero P. The hemostatic properties of chitosan in oral surgery. *Biomed Biotechnol Res J*. 2020;4(3):186–188. doi:10.4103/bbrj.bbrj_43_20
22. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71
23. Higgins JP, Altman DG, Gøtzsche PC, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*. 2011;343:d5928. doi:10.1136/bmj.d5928
24. Lewis SJ, Gardner M, Higgins J, et al. Developing the WCRF International/University of Bristol methodology for identifying and carrying out systematic reviews of mechanisms of exposure–cancer associations. *Cancer Epidemiol Biomarkers Prev*. 2017;26(11):1667–1675. doi:10.1158/1055-9965.EPI-17-0232
25. Ahn JC, Kang JW, Shin JI, Chung PS. Combination treatment with photodynamic therapy and curcumin induces mitochondria-dependent apoptosis in AMC-HN3 cells. *Int J Oncol*. 2012;41(6):2184–2190. doi:10.3892/ijo.2012.1661
26. Almalki Z, Algregri M, Alhosin M, Alkhaled M, Damiati S, Zamzami MA. In vitro cytotoxicity of curcuminoids against head and neck cancer HNO97 cell line. *Braz J Biol*. 2021;83:e248708. doi:10.1590/1519-6984.248708
27. Ardito F, Perrone D, Giuliani M, Testa NF, Lo Muzio L. Effects of curcumin on squamous cell carcinoma of tongue: An in vitro study. *Curr Top Med Chem*. 2018;18(3):233–243. doi:10.2174/1568026618666180412153824
28. Arun P, Sagayaray A, Azeem Mohiyuddin SM, Santosh D. Role of curcumin as an adjuvant in treatment of advanced head and neck squamous cell carcinoma. *Int J Otorhinolaryngol Head Neck Surg*. 2018;4(6):1388–1393. doi:10.18203/issn.2454-5929.ijohns20184348
29. Basak SK, Bera A, Yoon AJ, et al. A randomized, phase 1, placebo-controlled trial of APG-157 in oral cancer demonstrates systemic absorption and an inhibitory effect on cytokines and tumor-associated microbes. *Cancer*. 2020;126(8):1668–1682. doi:10.1002/cncr.32644
30. Camacho-Alonso F, López-Jornet P, Tudela-Mulero MR. Synergic effect of curcumin or lycopene with irradiation upon oral squamous cell carcinoma cells. *Oral Dis*. 2013;19(5):465–472. doi:10.1111/odi.12025
31. Chang PY, Peng SF, Lee CY, et al. Curcumin-loaded nanoparticles induce apoptotic cell death through regulation of the function of MDR1 and reactive oxygen species in cisplatin-resistant CAR human oral cancer cells. *Int J Oncol*. 2013;43(4):1141–1150. doi:10.3892/ijo.2013.2050
32. Essawy MM, Mohamed MM, Raslan HS, Rafik ST, Awaad AK, Ramadan OR. The theranostic potentialities of bioavailable nanocurcumin in oral cancer management. *BMC Complement Med Ther*. 2022;22(1):309. doi:10.1186/s12906-022-03770-3
33. De Souza Ferreira SB, Braga G, Oliveira ÉL, et al. Design of a nanostructured mucoadhesive system containing curcumin for buccal application: From physicochemical to biological aspects. *Beilstein J Nanotechnol*. 2019;10:2304–2328. doi:10.3762/bjnano.10.222
34. Ghosh S, Mallick S, Das U, et al. Curcumin stably interacts with DNA hairpin through minor groove binding and demonstrates enhanced cytotoxicity in combination with FdU nucleotides. *Biochim Biophys Acta Gen Subj*. 2018;1862(3):485–494. doi:10.1016/j.bbagen.2017.10.018
35. Gosangari SL, Watkin KL. Effect of preparation techniques on the properties of curcumin liposomes: Characterization of size, release and cytotoxicity on a squamous oral carcinoma cell line. *Pharm Dev Technol*. 2012;17(1):103–109. doi:10.3109/10837450.2010.522583
36. Jeon HS, Jo MH, Kim HJ, et al. Anticancer activities of diphenyl difluoroketone, a novel curcumin analog, on KB human oral cancer cells. *J Korean Soc Appl Biol Chem*. 2012;55:451–456. doi:10.1007/s13765-012-1168-8
37. Kim JY, Cho TJ, Woo BH, et al. Curcumin-induced autophagy contributes to the decreased survival of oral cancer cells. *Arch Oral Biol*. 2012;57(8):1018–1025. doi:10.1016/j.archoralbio.2012.04.005
38. Kötting C, Hofmann L, Lotfi R, et al. Immune-stimulatory effects of curcumin on the tumor microenvironment in head and neck squamous cell carcinoma. *Cancers (Basel)*. 2021;13(6):1335. doi:10.3390/cancers13061335
39. Kumbar VM, Muddapur U, Bin Muhsinah A, et al. Curcumin-encapsulated nanomicelles improve cellular uptake and cytotoxicity in cisplatin-resistant human oral cancer cells. *J Funct Biomater*. 2022;13(4):158. doi:10.3390/jfb13040158
40. Lai KC, Chueh FS, Hsiao YT, et al. Gefitinib and curcumin-loaded nanoparticles enhance cell apoptosis in human oral cancer SAS cells in vitro and inhibit SAS cell xenografted tumor in vivo. *Toxicol Appl Pharmacol*. 2019;382:114734. doi:10.1016/j.taap.2019.114734
41. Latimer B, Ekshyyan O, Nathan N, et al. Enhanced systemic bioavailability of curcumin through transmucosal administration of a novel microgranular formulation. *Anticancer Res*. 2015;35(12):6411–6418. PMID:26637850.

42. Liao F, Liu L, Luo E, Hu J. Curcumin enhances anti-tumor immune response in tongue squamous cell carcinoma. *Arch Oral Biol*. 2018;92:32–37. doi:10.1016/j.archoralbio.2018.04.015
43. Lin C, Tu C, Ma Y, et al. Curcumin analog EF24 induces apoptosis and downregulates the mitogen activated protein kinase/extracellular signal-regulated signaling pathway in oral squamous cell carcinoma. *Mol Med Rep*. 2017;16(4):4927–4933. doi:10.3892/mmr.2017.7189
44. Lin HY, Thomas JL, Chen HW, Shen CM, Yang WJ, Lee MH. In vitro suppression of oral squamous cell carcinoma growth by ultrasound-mediated delivery of curcumin microemulsions. *Int J Nanomedicine*. 2012;7:941–951. doi:10.2147/IJN.S28510
45. Lu Y, Ding N, Yang C, Huang L, Liu J, Xiang G. Preparation and in vitro evaluation of a folate-linked liposomal curcumin formulation. *J Liposome Res*. 2012;22(2):110–119. doi:10.3109/08982104.2011.627514
46. Ma C, Zhuang Z, Su Q, He J, Li H. Curcumin has anti-proliferative and pro-apoptotic effects on tongue cancer in vitro: A study with bioinformatics analysis and in vitro experiments. *Drug Des Devel Ther*. 2020;14:509–518. doi:10.2147/DDDT.S237830
47. Mazzarino L, Loch-Neckel G, Dos Santos Bubniak L, et al. Curcumin-loaded chitosan-coated nanoparticles as a new approach for the local treatment of oral cavity cancer. *J Nanosci Nanotechnol*. 2015;15(1):781–791. doi:10.1166/jnn.2015.9189
48. Mishra A, Kumar R, Tyagi A, et al. Curcumin modulates cellular AP-1, NF-kB, and HPV16 E6 proteins in oral cancer. *Ecancermedicalscience*. 2015;9:525. doi:10.3332/ecancer.2015.525
49. Mukherjee D, Dash P, Ramadass B, Mangaraj M. Nanocurcumin in oral squamous cancer cells and its efficacy as a chemo-adjuvant. *Cureus*. 2022;14(5):e24678. doi:10.7759/cureus.24678
50. Ohnishi Y, Sakamoto T, Zhengguang L, et al. Curcumin inhibits epithelial-mesenchymal transition in oral cancer cells via c-Met blockade. *Oncol Lett*. 2020;19(6):4177–4182. doi:10.3892/ol.2020.11523
51. Reiter M, Baumeister P, Boeck D, Schwenk-Zieger S, Harréus U. Reduction of DNA damage by curcumin and celecoxib in epithelial cell cultures of the oropharynx after incubation with tobacco smoke condensate. *Anticancer Res*. 2012;32(8):3185–3190. PMID:22843891.
52. Shin DS, Srivatsan E, Nasser H, et al. Potential role of botanical drug APG-157 as immune adjuvant in patients with oral squamous cell carcinoma. *J Clin Oncol*. 2019;37(15 Suppl):e17572. doi:10.1200/JCO.2019.37.15_suppl.e1757
53. Singh SP, Sharma M, Gupta PK. Enhancement of phototoxicity of curcumin in human oral cancer cells using silica nanoparticles as delivery vehicle. *Lasers Med Sci*. 2014;29(2):645–652. doi:10.1007/s10103-013-1357-7
54. Sivanantham B, Sethuraman S, Krishnan UM. Combinatorial effects of curcumin with an anti-neoplastic agent on head and neck squamous cell carcinoma through the regulation of EGFR-ERK1/2 and apoptotic signaling pathways. *ACS Comb Sci*. 2016;18(1):22–35. doi:10.1021/acscombsci.5b00043
55. Xi Y, Gao H, Callaghan MU, et al. Induction of BCL2-interacting killer, BIK, is mediated for anti-cancer activity of curcumin in human head and neck squamous cell carcinoma cells. *J Cancer*. 2015;6(4):327–332. doi:10.7150/jca.11185
56. Xiao C, Wang L, Zhu L, Zhang C, Zhou J. Curcumin inhibits oral squamous cell carcinoma SCC-9 cells proliferation by regulating miR-9 expression. *Biochem Biophys Res Commun*. 2014;454(4):576–580. doi:10.1016/j.bbrc.2014.10.122
57. Zhen L, Fan D, Yi X, Cao X, Chen D, Wang L. Curcumin inhibits oral squamous cell carcinoma proliferation and invasion via EGFR signaling pathways. *Int J Clin Exp Pathol*. 2014;7(10):6438–6446. PMID:25400722. PMCID:PMC4230161.
58. Liu T, Long T, Li H. Curcumin suppresses the proliferation of oral squamous cell carcinoma through a specificity protein 1/nuclear factor-κB-dependent pathway. *Exp Ther Med*. 2021;21(3):202. doi:10.3892/etm.2021.9635
59. Srivastava S, Mohammad S, Pant AB, et al. Co-delivery of 5-fluorouracil and curcumin nanohybrid formulations for improved chemotherapy against oral squamous cell carcinoma. *J Maxillofac Oral Surg*. 2018;17(4):597–610. doi:10.1007/s12663-018-1126-z
60. Fazli B, Irani S, Bardania H, Moosavi MS, Rohani B. Prophylactic effect of topical (slow-release) and systemic curcumin nano-niosome antioxidant on oral cancer in rat. *BMC Complement Med Ther*. 2022;22(1):109. doi:10.1186/s12906-022-03590-5
61. Alok A, Singh ID, Singh S, Jha A. Curcumin: Pharmacological actions and its role in head and neck squamous cell carcinoma – a review. *J Indian Acad Oral Med Radiol*. 2017;29(2):115–118. doi:10.4103/jiaomr.JIAOMR_100_16
62. Mansouri K, Rasoulpoor S, Daneshkhah A, et al. Clinical effects of curcumin in enhancing cancer therapy: A systematic review. *BMC Cancer*. 2020;20(1):791. doi:10.1186/s12885-020-07256-8
63. Borges GÁ, Rêgo DF, Assad DX, Coletta RD, De Luca Canto G, Silva Guerra EN. In vivo and in vitro effects of curcumin on head and neck carcinoma: A systematic review. *J Oral Pathol Med*. 2017;46(1):3–20. doi:10.1111/jop.12455
64. Zhu F, Tan G, Jiang Y, Yu Z, Ren F. Rational design of multi-stimuli-responsive gold nanorod-curcumin conjugates for chemo-photothermal synergistic cancer therapy. *Biomater Sci*. 2018;6(11):2905–2917. doi:10.1039/c8bm00691a
65. Madamsetty VS, Vazifehdoost M, Alhashemi SH, et al. Next-generation hydrogels as biomaterials for biomedical applications: Exploring the role of curcumin. *ACS Omega*. 2023;8(10):8960–8976. doi:10.1021/acsomega.2c07062
66. Mukherjee D, Krishnan A. Therapeutic potential of curcumin and its nanoformulations for treating oral cancer. *World J Methodol*. 2023;13(3):29–45. doi:10.5662/wjm.v13.i3.29
67. Shin MS, Yu JS, Lee J, et al. A hydroxypropyl methylcellulose-based solid dispersion of curcumin with enhanced bioavailability and its hepatoprotective activity. *Biomolecules*. 2019;9(7):281. doi:10.3390/biom9070281
68. Lamb SR, Wilkinson SM. Contact allergy to tetrahydrocurcumin. *Contact Dermatitis*. 2003;48(4):227. doi:10.1034/j.1600-0536.2003.00062.x
69. Lao CD, Ruffin MT 4th, Normolle D, et al. Dose escalation of a curcuminoid formulation. *BMC Complement Altern Med*. 2006;6:10. doi:10.1186/1472-6882-6-10
70. Panahi Y, Saadat A, Beiraghdar F, Hosseini Nouzari SM, Jalalian HR, Sahebkar A. Antioxidant effects of bioavailability-enhanced curcuminoids in patients with solid tumors: A randomized double-blind placebo-controlled trial. *J Funct Foods*. 2014;6:615–622. doi:10.1016/j.jff.2013.12.008

Review

Treatment methods for deep carious lesions in permanent teeth: A literature review

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Abstract

Complete removal of carious dentin is currently regarded as overtreatment, and the stepwise (two-step) or selective (one-step) caries removal procedures are recommended. This literature review was aimed to summarize research findings on deep caries lesion management approaches in permanent teeth, to determine which method provides better outcomes and to identify possible research gaps. Only intervention studies published between 2000 and 2023 in the English language were considered. The PubMed, Web of Science and Scopus databases were searched in September 2023, and 7 relevant records were considered for assessment. This literature review has shown that the number of studies comparing the methods of deep caries lesion management is limited, and the significant differences in protocols regarding the cavity depth, the endpoint of tissue excavation and the material used for cavity restoration indicate the need for the standardization of treatment. Both one- and two-stage caries removal significantly reduce the risk of pulp exposure in comparison with complete excavation; however, further clinical trials with long follow-up periods are necessary to determine which caries removal approach, stepwise or selective, provides better outcomes.

Keywords: permanent teeth, selective caries removal, stepwise caries removal, complete caries removal, deep caries treatment

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Highlights

- Stepwise caries removal (SwCR) and selective caries removal (SCR) are currently recommended for the management of deep carious lesions as alternatives to complete caries removal (CCR).
- This review included 7 studies published in English between 2000 and 2023, all of which evaluated at least 2 treatment approaches for deep carious lesions without pulp exposure in permanent teeth. Five studies compared complete vs. non-complete caries removal, while 2 studies directly compared SwCR and SCR.
- SwCR and SCR demonstrated clinical outcomes comparable to CCR, with no significant difference in the treatment success rates between the 2 non-complete caries removal techniques. Importantly, non-complete caries removal was associated with a lower risk of pulp exposure as compared to CCR.
- Considerable variation in clinical protocols was observed across studies, highlighting the need for standardized long-term clinical trials on treatment approaches for deep caries lesions in permanent dentition.

Introduction

The traditional approach to caries treatment is based on the concept of complete removal of carious dentin, leaving only hard dentin that meets tactile and color criteria.[1,2] The rationale for the above is to stop decay by eliminating bacteria, and to remove soft and demineralized dentin to provide a solid base for the restoration.[1,2] This approach has been used for decades and is still preferred by numerous dentists worldwide.[3,4] However, contemporary dentistry regards such cavity preparation as overtreatment.[5,6] Non-selective caries removal increases the risk of pulp exposure and pulp damage due to the diffusion of toxic compounds through the thin remaining dentin barrier over the pulpal wall. This contradicts one of the fundamentals of caries treatment, namely the preservation of pulp vitality.[7] In addition, non-selective caries removal may weaken the tooth structure and increase the tooth's susceptibility to damage.[2] Current evidence-based recommendations for the treatment of carious lesions state that it is not necessary to remove the entire carious tissue from the pulpal wall.[1,5] According to the minimal intervention dentistry (MID) guidelines, treatment should be restricted to the removal of the infected dentin only.[8] The remineralization of affected dentin can be achieved by the action of odontoblasts or by the diffusion of fluoride, calcium and phosphate ions from dental materials.[8]

The nomenclature and modern techniques for caries management have recently been discussed in the literature.[1,5,6,8–10] There are 2 main approaches: two- and one-step treatment. The 1st method – stepwise caries removal (SwCR) – comprises 2 stages. First, carious tissue is removed down to soft dentin and the cavity is filled with a temporary material with a remineralizing effect for a certain period of time. The amount of the removed carious tissue should be sufficient to allow the placement of a durable restoration.[5] During the re-entry visit, the cavity is reopened for the removal of the remaining decayed tissues up to firm dentin and the final filling is provided. The response of the pulp–dentin complex after treatment can be induced by the effect of dental materials (liners, temporary and permanent restorations) and several mechanisms, including the remineralization of dentin, the production of tertiary dentin and the reduction of post-operative pulpitis.[11,12] The 2nd approach – selective caries removal (SCR), also known as incomplete, partial or minimally invasive, consists in one-visit treatment. The idea is to leave carious (leathery/firm or soft) tissue on the pulpal wall and to seal the cavity with a permanent restoration. Both methods strictly require the preparation of external cavity walls up to sound enamel and hard dentin.[1,2,5,9]

Dental treatment should also be considered in terms of cost-effectiveness. There is evidence that the SCR approach saves up to 40% of costs and is associated with high success rates.[13–15] Stepwise excavation involves additional treatment costs, as a reopening visit is required; besides, some failure, such as a tooth fracture or damage to a temporary filling, may occur between treatment visits.[13] Schwendicke et al. found that most unfavorable outcomes in stepwise excavation were the result of the cavity reopening.[16]

The non-complete caries removal techniques were previously recommended for uncooperative and disabled children with severe dental caries, and as atraumatic restorative treatment (ART) in underdeveloped countries with a limited access to adequate dental equipment.[10] Recent research studies on the attitudes of dentists and dental students to deep carious lesion management show an increasing trend toward the implementation of modern approaches to the treatment of permanent teeth in the general population. On the other hand, those concepts have not yet been adopted by many dental practitioners; especially those with longer work experience are more likely to perform non-selective cavity preparation.[3,4,17,18]

There are several literature reviews on different aspects of incomplete carious dentin removal.[2,7,8,19–21] However, there is only one paper comparing the effectiveness of the stepwise and selective caries removal techniques,[21] and

no review specifically focuses on permanent dentition. Therefore, the aim of this review was to summarize research findings on deep caries lesion management approaches in permanent teeth and to identify possible research gaps.

Material and methods

A literature review was performed to assess how the research comparing different approaches to deep caries lesion management in permanent teeth has been conducted, which method provides better outcomes and what are the research gaps in this field. Two research questions were formulated: "Which approach, stepwise/selective caries removal vs. non-selective caries removal, provides better outcomes in permanent teeth?"; and "Which approach, stepwise caries removal vs. selective caries removal, provides better outcomes in permanent teeth?". The main criterion for including records in the present literature review was: articles published in peer-reviewed journals that described the results of at least 2 different treatment methods for deep carious lesions without pulp exposure in permanent dentition. Intervention studies published between 2000 and 2023 in the English language were considered. The exclusion criteria were as follows: non-research articles, such as protocols, books, proceedings, reviews, or letters to editors.

The keywords for search were developed by 3 authors (SB, KG and JB) based on a preliminary analysis of the literature. The following terms were used as a search strategy: ((((((((selective caries removal) OR (complete caries removal)) OR (partial caries removal)) OR (stepwise caries removal)) AND (permanent teeth)) OR (permanent dentition)) AND (deep caries)) NOT (case study)) NOT (review). The PubMed, Web of Science and Scopus databases were searched on September 8–9, 2023, to find relevant studies. This search was supplemented by the analysis of the reference lists of the retrieved articles and gray literature. The records were processed in EndNote Web (Clarivate™, London, UK) to remove duplicates. The results were analyzed by 3 authors (JB, KG and SB) according to the titles and abstracts to select relevant articles. Other authors (RŁ and ER) were asked for their opinion in case of doubts as to whether the article should be included in the analysis or not.

In the following step, the data for the analysis was collected using an Excel spreadsheet (Microsoft Office Professional Plus 2019; Microsoft Corporation, Redmond, USA). It comprised information on the authors and their affiliation (country), the year of publication, the study type, the number of cases, the patients' age, the tooth type, the criteria for caries depth assessment, the kind of intervention, the caries treatment procedure, the liner and the material used for restoration, the cavity re-entry period (if applicable), the outcome criteria, the follow-up period, and the authors' main conclusions. The form for data charting was developed by JB and RŁ, and then accepted by all authors. The analysis of the articles, the synthesis of the obtained results and data charting were carried out by 2 authors (KG and SB), and then another 2 authors (JB and ER) reviewed the records obtained.

Results

Figure 1 presents the literature search flow diagram. An initial search showed 216 articles (PubMed – 99, Web of Science – 62, Scopus – 55). Manual search did not reveal any additional articles. After examining the titles, 81 articles remained (PubMed – 35, Web of Science – 23, Scopus – 23). After applying the de-duplication function in the citation management software EndNote Web, 75 records were left. Based on the analysis of the abstracts, 11 manuscripts were selected for evaluation. However, after a detailed analysis of the content of the articles, 3 of them were excluded, since they concerned prospective studies, and their final results were presented in the papers by Bjørndal et al.[22] and Maltz et al.[23] One paper was excluded due to the evaluation of 2 protocols for the SCR method.[24]

The final number of records was 7 (Table 1).[22,23,25–29] Among the studies included in this review, 1 was a multicenter Scandinavian study,[22] 2 studies were from India,[25,28] and others were from Brazil,[23] Egypt,[26] Turkey,[27] and Pakistan.[29] All papers were published between 2017 and 2021. Five records compared modern caries removal approaches and CCR (Table 2).[22,25,27–29] Three of those studies applied the stepwise method[22,27,28] and in the other two, SCR was used.[25,29] Further 2 studies summarized in Table 3 compared one- and two-step caries removal with each other.[23,26]

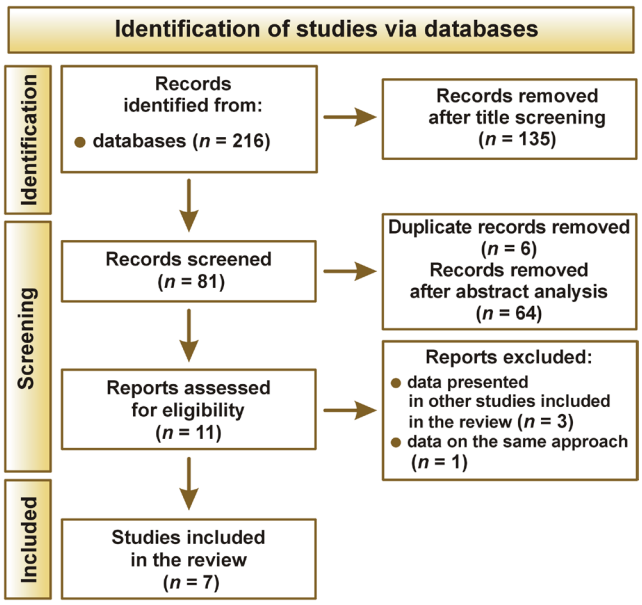

Fig. 1. Literature search flow diagram

Table 1. Main characteristics of the studies included in the analysis

Authors	Year of publication	Country	Study type	Intervention
Bjørndal et al.[22]	2017	Denmark/Sweden	RCT	SwCR vs. non-selective caries removal
Maltz et al.[23]	2018	Brazil	RCT	SCR vs. SwCR
Khokhar and Tewari[25]	2018	India	RCT	SCR vs. CCR
Labib et al.[26]	2019	Egypt	RCT	SCR vs. SwCR
Oz et al.[27]	2019	Turkey	RCT	SwCR vs. CCR
Manhas et al.[28]	2020	India	RCT	SwCR vs. indirect pulp capping after complete caries excavation to firm dentin
Ahmed et al.[29]	2021	Pakistan	RCT	SCR vs. CCR

RCT – randomized clinical trial; SwCR – stepwise caries removal; SCR – selective caries removal; CCR – complete caries removal.

Each study was conducted as a randomized clinical trial (RCT), but there were many differences in the study protocols. With regard to the patients' age, most studies were conducted on adults, but some included also the permanent teeth of young children (>6 years)[23] and teenagers (>14 years).[25] Another difference was the group of teeth – some studies were conducted exclusively on molars or on lateral teeth,[23,25,26,28,29] while other studies included whole dentition.[22,27] A deep carious lesion was diagnosed radiographically, but the definition of such a lesion varied from the involvement of ≥½ of the dentin layer to ¾ or more. One study defined a deep carious lesion as involving ⅔ of dentin.[26]

The protocols of intervention are described in detail in Tables 2 and 3. During complete carious dentin removal, the decayed tissues were removed to hard dentin with or without the use of Caries Detector dye, and then the tooth was restored with a glass-ionomer cement (GIC) base and a resin composite/amalgam filling.[22,25,27–29] Manhas et al. sealed the pulpal wall with calcium hydroxide, and followed with temporary restoration with zinc oxide–eugenol cement (ZOE).[28] At the revisit, calcium hydroxide was left on the pulpal wall and the tooth was restored with a resin composite.[28] Stepwise caries removal was performed by hand excavation or using a bur to soft dentin or firm dentin.[22,23,27,28] In this method, the remaining carious dentin was usually sealed with calcium hydroxide cement, and GIC or modified ZOE were used for a temporary filling. Only Labib et al. sealed the cavity directly with GIC.[26] The re-entry visit was carried out between 2 and 6 months, and it included the removal of the temporary filling, the complete removal of carious tissues (to hard or firm dentin) and the final filling of the cavity. In the SCR technique, most protocols consisted in removing the infected dentin down to a soft, moist layer on the pulpal wall, and the final restoration was made with GIC and a resin composite/amalgam.[23,25,26,29]

The follow-up period was 1 year, 18 months, 62 months, or 5 years.[22,23,25–29] The outcome criteria were based on the response of pulp and periapical tissues, and they included: a positive response to pulp testing; the absence of spontaneous pain and pain on palpation/percussion; the absence of fistulae/swelling; and a normal radiological image of the periapical area. The overall success rate of the stepwise method was higher or similar to that of CCR.[22,27,28] The selective removal of carious tissues showed a similar outcome as compared to teeth with complete excavation (after excluding teeth with pulp exposure during excavation).[25,29] However, in the long-term observations lasting around 5 years, the effectiveness rate of SwCR was lower than in short-term observations, and ranged, after excluding teeth with uncompleted treatment, between 60.2% and 75%.[22,23] Taking into account stepwise and selective excavation, the studies showed comparable results.[23,26] Maltz et al. concluded that the effectiveness of the stepwise technique was highly influenced by the cases without complete treatment.[23] In all the studies reporting the level of pulp exposure, both one- and two-stage caries removal significantly reduced the risk of such events in comparison with complete excavation.[22,25,28,29]

Discussion

This literature review showed that the number of studies comparing different methods of removing carious dentin in permanent dentition is limited. The evaluated studies varied in terms of important factors that may influence the outcome (the patient's age, the threshold of deep caries, the restorative method, the observation period). However, all records showed that the stepwise and selective approaches had a success rate at least comparable to the traditional caries removal technique. There were fewer cases of pulp exposure during one- and two-step caries removal than during complete excavation. On the other hand, due to a small number of records, no clear conclusion could be drawn as to which method, the one- or two-stage approach, is more beneficial for permanent dentition. No statistically significant differences in the success rates were found in the studies comparing modern approaches. Therefore, there is a gap in research on the preferred treatment method, and more clinical trials with long follow-up periods should be conducted. However, there are opinions in the literature that one-step incomplete excavation reduces the risk of failure as compared to two-step removal.[16]

Table 2. Summary of studies comparing complete caries removal (CCR) with stepwise caries removal (SwCR) or selective caries removal (SCR)

Author	No. of cases/ patients' age [years]	Tooth type	Caries depth assessment	Intervention/liner and restoration material	Cavity re-entry period	Outcome criteria	Follow-up period	Success rate/ pulp exposure rate	Main conclusions
Bjørndal et al.[22]	314 cases >18	whole dentition	radio-graphically ≥¾ dentin	SwCR: hand excavation to soft dentin on the pulpal wall, indirect pulp capping with calcium hydroxide cement and a temporary filling with GIC; during the re-entry visit, carious tissues were removed to the discolored but hard dentin, and the tooth was restored with a calcium hydroxide base and a resin composite CCR: the removal of carious tissues to the discolored but hard dentin, a calcium hydroxide base and a temporary filling with GIC; during the re-entry visit, the temporary filling was removed, but not the base, and cavity restoration was performed with a resin composite	8–12 weeks 8–12 weeks	– unexposed pulp – a positive response to the cold/electrical tests – the absence of apical radiolucency	5 years	success rate: SwCR: 60.2% CCR: 46.3% pulp exposure: SwCR: 21.2% CCR: 35.5%	After 5 years, SwCR showed higher success rates as compared to CCR with regard to pulp vitality and the lack of periapical lesions. SwCR resulted in less pulp exposure cases than CCR. For teeth with non-exposed pulp, the patient's age had no influence on the success rate.
Khokhar and Tewari[25]	143 cases 25.9 (14–54)	molars	radio-graphically ≥ ½ dentin	SCR: cavity preparation with low speed metal burs and/or a hand excavator to soft, wet, carious dentin on the pulpal wall, resin-modified GIC applied to the pulpal wall, the tooth restored with a resin composite CCR: cavity preparation with low speed metal burs and/or a hand excavator with the aid of Caries Detector dye until dentin stopped getting stained, resin-modified GIC applied to the pulpal wall, the tooth restored with a resin composite	none	– a positive response to the cold/electrical tests – the absence of spontaneous pain, fistulae or swelling – the absence of radiolucency at the furcal/ periapical region	18 months	success rate: SCR: 92.5% CCR: 98.2% (excluding teeth with pulp exposure) pulp exposure: SCR: 0.0% CCR: 18.4%	Less pulp exposure occurred in the SCR group than in the CCR group. The were no statistically significant differences in the success rates between the SCR and CCR groups.
Oz et al.[27]	281 cases 18–60	whole dentition	radio-graphically ≥¾ dentin	SwCR: the hand excavation of the soft necrotic carious tissue to skin like, scaly caries, calcium hydroxide and GIC as temporary restoration; during the re-entry visit, the remaining carious dentin was removed with an excavator or a bur, calcium hydroxide paste and GIC were used as a base, and the tooth was restored with a composite resin/amalgam CCR: caries removal, calcium hydroxide paste and GIC were used as a base, and the tooth was restored with a composite resin/amalgam	6 months	– asymptomatic teeth – teeth without clinical and radiographic evidence of periapical lesions	62 months (on average)	success rate: SwCR: 85.7% CCR: 90.9% pulp exposure: N/A	SwCR and CCR showed comparable results.
Manhas et al.[28]	88 cases N/A	molars	radio-graphically ≥¾ dentin	SwCR: hand excavation to soft dentin on the pulpal wall, sealing dentin with calcium hydroxide, ZOE as temporary restoration; during the re-entry visit, dentin was removed with a hand instrument, and the tooth was restored with calcium hydroxide and a resin composite CCR to firm dentin: hand excavation to a thin layer of the affected dentin left on the pulpal wall, sealing dentin with calcium hydroxide, ZOE as temporary restoration; during the re-entry visit, calcium hydroxide was left on the pulpal wall and the tooth was restored with a resin composite	2 months	– clinical and radiological symptoms of pulpal involvement or periapical pathology	1 year	success rate: SwCR: 97.3% CCR: 82.4% pulp exposure: SwCR: 0.0% CCR: 11.3%	SwCR presented higher success rates than CCR.
Ahmed et al.[29]	70 cases 23.4 ±5.5 25.6 ±4.9	molars	radio-graphically ≥½ dentin	SCR: the removal of superficial necrotic dentin with a low-speed bur to the wet layer left on the pulpal wall, resin-modified GIC applied to the pulpal wall, the tooth restored with a resin composite CCR: the removal of carious tissues confirmed by Caries Detector dye, resin-modified GIC and a resin composite	none	– the absence of irreversible pulpitis – the absence of periapical inflammation	18 months	success rate: SCR: 93.3% CCR: 100.0% pulp exposure: SCR: 0.0% CCR: 23.3%	There was no statistically significant difference in the success rates between SCR and CCR. SCR showed a significantly lower risk of pulp exposure as compared to CCR.

GIC – glass-ionomer cement; ZOE – zinc oxide–eugenol cement; N/A – data not available.

Table 3. Summary of studies comparing stepwise caries removal (SwCR) and selective caries removal (SCR)

Author	No. of cases/ patients' age [years]	Tooth type	Caries depth assessment	Intervention/liner and restoration material	Cavity re-entry period	Outcome criteria	Follow-up period	Success rate/ pulp exposure rate	Main conclusions
Maltz et al.[23]	299 cases 6–53	permanent molars	radio-graphically ≥½ dentin	SCR: hand excavation to soft tissue on the pulpal wall, tooth restoration with GIC and a resin composite/amalgam SwCR: the hand excavation of disorganized soft tissue from the pulpal wall, indirect pulp capping with calcium hydroxide cement and a temporary filling with modified ZOE; during the re-entry visit, the removal of the remaining caries, and tooth restoration with GIC and a resin composite/amalgam	90 days	– a positive response to the cold test – a negative response to percussion – the absence of spontaneous pain – the absence of periapical lesions	5 years	success rate: SCR: 80% SwCR: 75% (for teeth with complete treatment) and 56% (including teeth without complete treatment) pulp exposure: N/A	SCR and SwCR showed similar success rates, assuming the patient from the SwCR group returned to complete treatment. The lower effectiveness of SwCR was due to the non-completion of the treatment protocol in some patients who did not attend the re-opening visit.
Labib et al.[26]	132 cases 18–47	premolars/ molars	radio-graphically ≥⅔ dentin	SCR: soft dentin left on the pulpal/axial wall, tooth restoration with GIC during the first visit, GIC partially removed to the level of a liner, tooth restoration with a composite resin SwCR: soft dentin left on the pulpal/axial wall, tooth restoration with GIC during the first visit; during the re-entry visit, caries excavation to firm dentin, tooth restoration with GIC as a liner and a composite resin	3–4 months	– a positive response to the cold test – the absence of spontaneous pain – a negative response to percussion – the lack of sinus tracts/swelling	1 year	success rate: SCR: 89.4% SwCR: 84.9% pulp exposure: SCR: 0.0% SwCR: 7.6%	There were no statistically significant differences in the success and survival rates between SCR and SwCR.

N/A – data not available.

The depth of carious lesions should be taken into account when choosing the treatment method, since the dentin thickness and permeability can influence the outcome. However, a clinical assessment of the residual dentin thickness is difficult.[7] Recent recommendations define deep caries as a lesion reaching the inner third or quarter of dentin on radiographs, with the risk of pulp exposure.[5] The European Society of Endodontology (ESE) has proposed a division into deep and very deep lesions.[30] The former are diagnosed when a carious lesion reaches up to a quarter of dentin on a radiograph, but a zone of radiopaque dentin separating the demineralized tissue from pulp can be found. Very deep caries means the presence of demineralized dentin throughout the thickness of the pulpal wall, with a high probability of bacterial invasion within tertiary dentin and even pulp. Thus, finding features of a very deep cavity on a radiographic examination may be a disqualifying factor in choosing the SCR method.[7] All the studies included in the present review used the radiographic evaluation to diagnose deep caries, but authors established different thresholds – from ½ to ¾ of the dentin layer, which made it difficult to compare the effectiveness of these methods.[22,23,25–29]

Other differences between the evaluated studies comprised the method for removing carious dentin, the endpoints of cavity preparation (soft or firm dentin) and the restoring method. So far, no consensus has been reached as to where to stop tissue excavation and which material is most suitable for filling the cavity during non-invasive caries removal.[31] However, the hand excavation of decomposed dentin on the pulpal wall seems to be more appropriate. Mechanical removal with round metal burs leads to excessive cavity preparation. Lim et al. emphasized that the determination of the level of removal of the involved dentin should be based on the dentin texture rather than its color.[6] The amount of the excavated infected tissue is one of the major concerns of the partial caries removal approach. According to Maltz et al., the recommended method for deep carious lesions is selective removal to soft dentin.[1] The evaluated studies confirmed that soft dentin did not have to be completely removed from the pulpal wall to maintain a long-term positive outcome.

In the analyzed studies, the products containing calcium hydroxide were mainly used in the stepwise technique. The sealing of carious dentin with calcium hydroxide reduces the number of microorganisms even below the level found in dentin after the conventional caries removal.[12] However, according to a recent recommendation, there is no need to seal soft dentin with cavity liners.[1] In contrast, the cavities treated by non-selective caries removal were usually restored with the combination of GIC and a resin composite. Glass-ionomer cement has a well-known high remineralization potential. Other materials, such as mineral trioxide aggregate (MTA), BioCeramic, silver diamine fluoride, propolis, or hesperidin, were tested as cavity

sealers in SCR.[32,33] However, Gözetici-Çil et al. found that calcium silicate cement used in SCR to soft dentin provided no additional benefit.[24]

The tooth's resistance to occlusal forces was not investigated in the studies included in this review. Undoubtedly, this aspect should be taken into account when planning treatment. Leaving non-hard dentin can be a factor affecting the biomechanical properties of the tooth tissue and restoration. There are reports stating that teeth after SCR have lower fracture resistance and increased deflection of cusps; therefore, restorative materials that can support the tooth structure should be chosen.[34] On the other hand, Scholz et al. found that SCR did not impair the marginal integrity of the restoration.[35]

Limitations

This review has some limitations. Only studies comparing at least 2 different approaches were included; therefore, some publications confirming the advantages of stepwise or selective caries removal were excluded from the analysis. Papers evaluating outcomes in primary dentition were not included. The decision to exclude them from the assessment was based on the fact that the risk of failure is higher for deciduous teeth due to difficulty in proper sealing and the short lifespan of deciduous teeth, which excludes the possibility of a long-term evaluation.[1] The small number of studies available for comparison and differences in the study design highlight the need for standardized long-term clinical trials on treatment approaches for deep caries lesions in permanent dentition.

Conclusions

This literature review has shown that the number of studies comparing the methods of deep caries lesion management is limited, and the significant differences in protocols regarding the cavity depth, the endpoint of tissue excavation and the material used for cavity restoration indicate the need for the standardization of treatment. Further clinical trials with long follow-up periods are necessary to determine which caries removal approach, stepwise or selective, provides better outcomes.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Maltz M, Jardim JJ, Alves LS. Chapter 9.3: Current concepts of caries removal in daily practice. *Monogr Oral Sci*. 2023;31:172–187. doi:10.1159/000530613
2. Barrett B, O'Sullivan M. Management of the deep carious lesion: A literature review. *J Ir Dent Assoc*. 2021;67(1):36–42. https://dentist.ie/app/uploads/2025/12/JIDA_FebMar2021_NonMember.pdf. Accessed September 4, 2023.
3. Schwendicke F, Stangvaltaite L, Holmgren C, et al. Dentists' attitudes and behaviour regarding deep carious lesion management: A multi-national survey. *Clin Oral Investig*. 2017;21(1):191–198. doi:10.1007/s00784-016-1776-5
4. Chai B, Tay B, Chow C, Fuss J, Krishnan U. Treatment preferences for deep caries lesions among Australian dentists. *Aust Dent J*. 2020;65(1):83–89. doi:10.1111/adj.12740
5. Innes NP, Frencken JE, Bjørndal L, et al. Managing carious lesions: Consensus recommendations on terminology. *Adv Dent Res*. 2016;28(2):49–57. doi:10.1177/0022034516639276
6. Lim ZE, Duncan HF, Moorthy A, McReynolds D. Minimally invasive selective caries removal: A clinical guide. *Br Dent J*. 2023;234(4):233–240. doi:10.1038/s41415-023-5515-4
7. Bjørndal L, Simon S, Tomson PL, Duncan HF. Management of deep caries and the exposed pulp. *Int Endod J*. 2019;52(7):949–973. doi:10.1111/iej.13128
8. Frencken JE, Peters MC, Manton DJ, Leal SC, Gordan VV, Eden E. Minimal intervention dentistry (MID) for managing dental caries – a review: Report of a FDI task group. *Int Dent J*. 2012;62(5):223–243. doi:10.1111/idj.12007
9. Machiulskiene V, Campus G, Carvalho JC, et al. Terminology of dental caries and dental caries management: Consensus report of a workshop organized by ORCA and Cariology Research Group of IADR. *Caries Res*. 2020;54(1):7–14. doi:10.1159/000503309
10. Jodłowska A, Leś-Smolarczyk A, Postek-Stefańska L. Description of five deep caries management methods and their use in contemporary dentistry. *J Stoma*. 2018;71(3):302–307. doi:10.5114/jos.2018.80639
11. Widbiller M, Weiler R, Knüttel H, Galler KM, Buchalla W, Scholz KJ. Biology of selective caries removal: A systematic scoping review protocol. *BMJ Open*. 2022;12(2):e061119. doi:10.1136/bmjopen-2022-061119
12. Maltz M, Henz SL, De Oliveira EF, Jardim JJ. Conventional caries removal and sealed caries in permanent teeth: A microbiological evaluation. *J Dent*. 2012;40(9):776–782. doi:10.1016/j.jdent.2012.05.011
13. Jardim JJ, Alves LS, Decourt RF, De Paula LM, Mestrinho HD, Maltz M. Cost-effectiveness of selective caries removal versus stepwise excavation for deep caries lesions. *Braz Oral Res*. 2023;37:e083. doi:10.1590/1807-3107bor-2023.vol37.0083
14. Schwendicke F, Stolpe M, Meyer-Lueckel H, Paris S, Dörfer CE. Cost-effectiveness of one- and two-step incomplete and complete excavations. *J Dent Res*. 2013;92(10):880–887. doi:10.1177/0022034513500792
15. Schwendicke F, Paris S, Stolpe M. Cost-effectiveness of caries excavations in different risk groups – a micro-simulation study. *BMC Oral Health*. 2014;14:153. doi:10.1186/1472-6831-14-153

16. Schwendicke F, Meyer-Lueckel H, Dörfer C, Paris S. Failure of incompletely excavated teeth – a systematic review. *J Dent*. 2013;41(7):569–580. doi:10.1016/j.jdent.2013.05.004
17. Kotha SB, Binhuwaishel HA, Almuhaydib RN, Alzeghaibi LY, Alhajri MA. Clinical decision-making in managing deep carious lesions in primary teeth based on clinical experience among pediatric dentists – a cross-sectional study. *J Popul Ther Clin Pharmacol*. 2022;28(2):e17–e28. doi:10.47750/jptcp.2022.860
18. Othman NI, Ismail HU, Mohammad N, Ghazali N, Alauddin MS. An evaluation on deep caries removal method and management performed by undergraduate dental students: A Malaysia experience. *Eur J Dent*. 2021;15(2):281–289. doi:10.1055/s-0040-1721546
19. Hoefler V, Nagaoka H, Miller CS. Long-term survival and vitality outcomes of permanent teeth following deep caries treatment with step-wise and partial-caries-removal: A systematic review. *J Dent*. 2016;54:25–32. doi:10.1016/j.jdent.2016.09.009
20. Frota Barros MM, De Queiroz Rodrigues MI, Gomes Muniz FW, Azevedo Rodrigues LK. Selective, stepwise, or nonselective removal of carious tissue: Which technique offers lower risk for the treatment of dental caries in permanent teeth? A systematic review and meta-analysis. *Clin Oral Investig*. 2020;24(2):521–532. doi:10.1007/s00784-019-03114-5
21. Yao Y, Luo A, Hao Y. Selective versus stepwise removal of deep carious lesions: A meta-analysis of randomized clinical trials. *J Dent Sci*. 2023,18(1):17–26. doi:10.1016/j.jds.2022.07.021
22. Bjørndal L, Fransson H, Bruun G, et al. Randomized clinical trials on deep carious lesions: 5-year follow-up. *J Dent Res*. 2017;96(7):747–753. doi:10.1177/0022034517702620
23. Maltz M, Koppe B, Jardim JJ, et al. Partial caries removal in deep caries lesions: A 5-year multicenter randomized controlled trial. *Clin Oral Investig*. 2018;22(3):1337–1343. doi:10.1007/s00784-017-2221-0
24. Gözetici-Çil B, Erdem-Hepşenoğlu Y, Tekin A, Özcan M. Selective removal to soft dentine or selective removal to firm dentine for deep caries lesions in permanent posterior teeth: A randomized controlled clinical trial up to 2 years. *Clin Oral Investig*. 2023;27(5):2125–2137. doi:10.1007/s00784-022-04815-0
25. Khokhar M, Tewari S. Outcomes of partial and complete caries excavation in permanent teeth: A 18 month clinical study. *Contemp Clin Dent*. 2018;9(3):468–473. doi:10.4103/ccd.ccd_344_18
26. Labib ME, Hassanein OE, Moussa M, Yassen A, Schwendicke F. Selective versus stepwise removal of deep carious lesions in permanent teeth: A randomised controlled trial from Egypt – an interim analysis. *BMJ Open*. 2019;9(9):e030957. doi:10.1136/bmjopen-2019-030957
27. Oz FD, Bolay S, Bayazit EO, Bicer CO, Isikhan SY. Long-term survival of different deep dentin caries treatments: A 5-year clinical study. *Niger J Clin Pract*. 2019;22(1):117–124. doi:10.4103/njcp.njcp_370_18
28. Manhas S, Pandit IK, Gugnani N, Gupta M. Comparative evaluation of the efficacy of stepwise caries excavation vs indirect pulp capping in preserving the vitality of deep carious lesions in permanent teeth of pediatric patients: An in vivo study. *Int J Clin Pediatr Dent*. 2020;13(Suppl 1):S92–S97. doi:10.5005/jp-journals-10005-1874
29. Ahmed MR, Aaslam S, Bukhari JH. Comparison of partial and complete caries excavation in permanent teeth: An 18 months follow-up. *Pak J Med Health Sci*. 2021;15(5):1214–1216. doi:10.53350/pjmhs211551214
30. Duncan HF, Galler KM, Tomson PL, et al.; European Society of Endodontology (ESE). European Society of Endodontology position statement: Management of deep caries and the exposed pulp. *Int Endod J*. 2019;52(7):923–934. doi:10.1111/iej.13080
31. Uribe S. Partial caries removal in symptomless teeth reduces the risk of pulp exposure. *Evid Based Dent*. 2006;7(4):94. doi:10.1038/sj.ebd.6400444
32. Ali A, Almaroof A, Festy F, Banerjee A, Mannocci F. In vitro remineralization of caries-affected dentin after selective carious tissue removal. *World J Dent*. 2018;9(3):170–179. doi:10.5005/jp-journals-10015-1529
33. Anani H, Elasser D, Niazy M, Jamil W, Elsharkawy D. Evaluation of the remineralization and antibacterial effect of natural versus synthetic materials on deep carious dentin: A randomized controlled trial. *Dent Med Probl*. 2023;60(1):87–97. doi:10.17219/dmp/147075
34. Corral-Nuñez C, Meyer-Lueckel H, Araya-Cabello I, Martin J, Estay J, Wierichs RJ. Mechanical properties of teeth restored after selective 2021;15(1):204–212. https://www.scielo.cl/pdf/ijodontos/v15n1/0718-381X-ijodontos-15-01-204.pdf. Accessed November 11, 2023.
35. Scholz KJ, Hinderberger M, Widbiller M, Federlin M, Hiller KA, Buchalla W. Influence of selective caries excavation on marginal penetration of class II composite restorations in vitro. *Eur J Oral Sci*. 2020;128(5):405–414. doi:10.1111/eos.12726

Research letter

Exploring the association between cervico-cranio-mandibular musculoskeletal function and obstructive sleep apnea: A preliminary study

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Abstract

Background. Obstructive sleep apnea (OSA) is a prevalent chronic condition characterized by recurrent episodes of upper airway narrowing or collapse during sleep. Obstructive sleep apnea is associated with various health issues, including cardiovascular disease and metabolic disorders. The cervico-cranio-mandibular complex (CCMC) plays a critical role in musculoskeletal functions such as mouth opening and head stabilization; however, its relationship with OSA remains insufficiently explored.

Objectives. The aim of the study was to evaluate the association between cervico-cranio-mandibular musculoskeletal function and OSA severity, based on the hypothesis that dysfunctions within the CCMC are associated with greater OSA severity.

Material and methods. A pilot cross-sectional study was conducted involving 32 patients with symptoms of OSA. The participants underwent assessment of cervico-cranio-mandibular musculoskeletal function and an ambulatory sleep study to determine OSA severity. Spearman's tests were used to analyze the relationships between cervico-cranio-mandibular musculoskeletal parameters and OSA severity.

Results. Significant negative correlations were observed between cervical range of motion (ROM) (flexion, left rotation, right/left lateral flexion) and OSA severity. The strongest correlation was identified for cervical movement in the frontal plane ($r = -0.607$, $p < 0.001$), especially among females ($r = -0.772$, $p < 0.01$). A positive relationship was found between mouth-opening muscle endurance and OSA severity in male participants ($r = 0.445$, $p < 0.05$).

Conclusions. This preliminary study highlights potential associations between specific musculoskeletal characteristics of the CCMC and OSA severity, with notable sex-specific patterns. These findings may contribute to the development of targeted screening approaches and support individualized physiotherapeutic interventions.

Keywords: neck pain, posture, temporomandibular joint, obstructive sleep apnea, craniomandibular disorders

Highlights

- A significant negative correlation was identified between cervical range of motion and the severity of obstructive sleep apnea (OSA).
- Sex-specific differences were found in the association between cervico-cranio-mandibular function and OSA.
- There is a positive correlation between mouth-opening muscle endurance and OSA severity in male participants.
- Cervico-cranio-mandibular physiotherapy represents a potential novel, non-invasive management approach for OSA.
- Musculoskeletal assessment and research may contribute to a better understanding of OSA pathophysiology.

Introduction

Obstructive sleep apnea (OSA) is a common and underdiagnosed condition characterized by recurrent episodes of upper airway obstruction during sleep, leading to intermittent hypoxia, sleep fragmentation and elevated sympathetic nervous system activity.[1] These disruptions contribute to the development of cardiovascular disease,[2] metabolic disorders,[3] cognitive impairments,[4] and reduced quality of life.[5] Obstructive sleep apnea is strongly associated with hypertension, atrial fibrillation, type 2 diabetes mellitus, stroke, and myocardial infarction.[6]

While anatomical factors such as obesity, macroglossia, retrognathia, malocclusion, hyoid position, and tonsillar hypertrophy are well-established contributors to OSA,[1,7–9] the role of musculoskeletal function in maintaining upper airway patency remains less understood. The cervico-cranio-mandibular complex (CCMC), including the cervical spine, mandible and associated musculature, may influence airway stability through biomechanical and neuromuscular mechanisms. For example, cervical spine posture can affect pharyngeal wall tension,[8] while masticatory muscle activity may alter hyoid bone and tongue positioning.[1,7,10]

Dysfunction or imbalance within the CCMC may compromise airway patency, even in the absence of overt anatomical abnormalities. Yet, clinical research on the musculoskeletal contribution to OSA is limited.[11,12] In light of this gap, conservative interventions such as physical therapy may offer potential therapeutic value.

Furthermore, sex-related differences in craniofacial anatomy, fat distribution and neuromuscular control suggest the presence of distinct OSA phenotypes. Women may rely more on accessory musculature and often present with atypical symptoms.[10]

The present pilot study investigated whether the neuromuscular function and mobility of the CCMC are associated with the severity of OSA. We hypothesized that reduced cervical mobility, altered posture, and imbalances in jaw muscle function would correlate with higher apnea–hypopnea index (AHI) values, with possible sex-based differences.

Material and methods

Study design and participants

This pilot cross-sectional observational study was conducted between August 2023 and May 2024. A convenience sample of 32 participants (20 males, 12 females), aged 18–70 years, was recruited through referrals from an otolaryngologist based on clinical suspicion of OSA. All participants underwent a comprehensive musculoskeletal evaluation followed by an ambulatory sleep study.

The inclusion criteria were as follows: 18–70 years of age (adult population); body mass index (BMI) ≤32 (to minimize a clear confounding factor); and referral for an overnight sleep study due to suspected OSA (e.g., excessive daytime sleepiness, loud snoring, morning headaches, unrefreshing sleep, or frequent nocturnal arousals). The exclusion criteria included prior treatment for OSA, a diagnosis of temporomandibular disorders based on the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD),[13] subjective complaints of significant neck pain within the past 30 days, surgical interventions involving the CCMC, or neurological conditions affecting motor control. Participants who had received recent Botox treatment for bruxism or experienced technical issues during the sleep study were also excluded.

The study was approved by the Helsinki Committee of Soroka Medical Center, Ben-Gurion University of the Negev, Be'er-Sheva, Israel (approval No. SOR-0183-22). Written informed consent was obtained from all participants. A reliability protocol was implemented for mouth-opening and mouth-closing muscle performance measurements. The study protocol was retrospectively registered in the Open Science Framework (OSF) (https://doi.org/10.17605/OSF.IO/RFN75).

Musculoskeletal evaluation of the cervico-cranio-mandibular complex

Two trained physiotherapists (AW and DG) performed all physical assessments. Both examiners were blinded to the results of the sleep study to minimize assessment bias.

Mouth opening

Isometric strength during mandibular depression and protrusion was assessed using a novel, adjustable extraoral force measurement device developed by the research team (Fig. 1). The device incorporated 2 piezoresistive force transducers (FMS-2X1002-15K-R3205w; Vishay, Chartres, France), each with a maximum load of 150 N, providing digital force output in real time. In the present study, intra- and inter-rater reliability of the device were evaluated.

The participants were seated upright with full back support and fitted with the device adjusted to allow 20 mm of mouth opening. Based on a previous protocol,[14] maximal pain-free isometric mouth-opening strength was measured 3 times. The maximal force achieved in 3 s was recorded and averaged. The verbal instruction for the participant was: "On the count of three, open your mouth as wide as you can". Afterward, based on the same protocol,[14] an endurance test was performed. The participants were required to maintain 30% of their maximal force (to fatigue the mouth openers) using screen-based biofeedback (±10% margin). For instance, for a maximal force of 10 kg, the endurance target was 3 kg (range 2–4 kg). The maximal pain-free time duration within this target range was recorded using a stopwatch, following a brief practice period. The verbal instruction was: "Maintain your mouth open at this target level as long as you can".

Mouth closing

Based on a previous protocol,[14] bite force was measured using a piezoresistive transducer-based device (iBite Pro®; Loadstar® Sensors, Fremont, USA), with a maximum load of 1,000 N and digital output displayed in real time.

The participants were seated upright with full back support. Maximal pain-free bite force was recorded over 3 trials and averaged. An endurance test was followed to assess fatiguability, during which participants maintained 30% of their maximal bite force using screen-based biofeedback (±10% margin). After a brief practice, the pain-free endurance time within the target range was measured using a stopwatch. Final outcomes included maximal strength [kg] and endurance time [s] for both mouth-opening and mouth-closing tests.

Reliability assessment for mouth-opening and mouth-closing tests

Intra-rater reliability was assessed by repeating the measurements on the following day by the same examiner, while inter-rater reliability involved 2 examiners performing measurements 1 h apart.

Neck muscle performance

The neck flexor muscle endurance test assessed craniocervical flexor function.[15] Based on the protocol,[15] the assessment was performed in a hook-lying position. The participants were instructed to retract their chin, lift their head approx. 2.5 cm above the surface while maintaining the chin tuck, and hold the position as long as possible. The test ended when the chin tuck was lost or when the head rested on the examiner's hand for more than 1 s.

Cervical range of motion

Cervical range of motion (ROM), which is often altered in association with cervical disorders,[16] was measured using a cervical ROM device (Performance Attainment Associates, Roseville, USA), a commonly used tool for the measurement of cervical spine mobility.[17] The participants were seated upright with the trunk supported against the backrest and feet flat on the floor. Six physiological movements, namely flexion, extension, right and left lateral flexion, and right and left rotation, were assessed in a randomized order. Each movement was repeated 3 times with 30-s rest intervals. The mean value of the 3 measurements, expressed in degrees, was recorded for each direction.

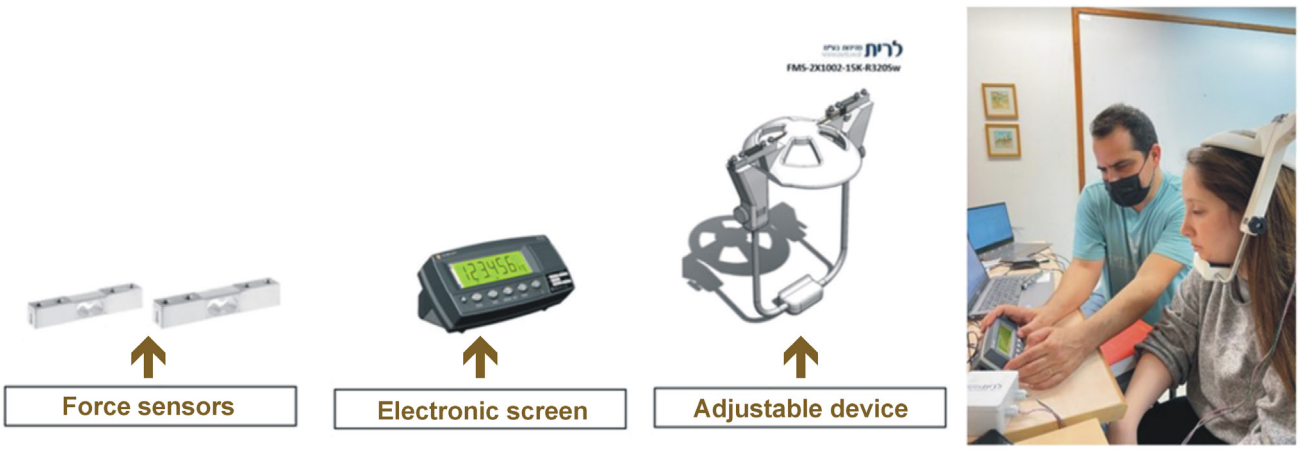

Fig. 1. Adjustable extraoral force measurement device (parts of the figure reproduced with permission from Larit Measurements Ltd.)

Flexion–rotation test

The flexion–rotation test (FRT)[18] was used to assess upper cervical (C1–C2) mobility. In the supine position, the participants performed maximal head rotation to each side while maintaining full cervical flexion. Range limitations and asymmetries were documented.

Assessment of head and neck posture

The assessment of head and neck posture was conducted using craniocervical angle analysis based on standardized lateral photographs.[19]

Sleep study

The severity of OSA was assessed using a validated home sleep testing device (WatchPAT® 300; ZOLL® Itamar®, Caesarea, Israel) that records peripheral arterial tone (PAT), heart rate and oxygen saturation.[20] The main outcome was the AHI, representing the number of apneas and hypopneas per hour of sleep. The participants wore the device overnight at home, which consisted of a wrist unit, a finger probe and a chest sensor. The data was interpreted by the Sleep-Awake Laboratory of Soroka Medical Center, Ben-Gurion University of the Negev, Be'er-Sheva, Israel.

Statistical analysis

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA). Descriptive statistics were calculated as mean (*M*) and standard deviation (*SD*) values. The Shapiro–Wilk test was used to assess normality of data distribution. One-way analysis of variance (ANOVA) was utilized to compare demographic and CCMC variables across OSA severity groups. Spearman's correlation coefficients were calculated to evaluate associations between CCMC function and OSA severity. Effect sizes were interpreted according to Cohen's guidelines for Pearson's *r*, as follows: 0.10 (small); 0.30 (medium); and 0.50 (large). Intra- and inter-rater reliability for mouth-opening and mouth-closing muscle measurements were evaluated using intraclass correlation coefficients (*ICC*s), based on a two-way mixed-effects model (consistency type) and 95% confidence intervals (95% *CI*s). The level of statistical significance was set at $p < 0.05$. As an exploratory study, the sample size of 32 participants was considered consistent with pilot study recommendations prioritizing feasibility and trend identification over statistical power.[21]

Results

The study included 32 participants (20 males and 12 females). The mean age was 47.4 ±14.7 years (range: 22–68 years), and the mean BMI was 26.75 ±2.53 kg/m^2 (range: 22.9–31.9 kg/m^2). Based on the AHI values, 28% of the participants were classified as having mild OSA, 41% as having moderate OSA, 12% as having severe OSA, while 19% had AHI values <5. All outcome measures were successfully obtained for all participants, ensuring a complete dataset for analysis.

Mouth-opening muscle strength and endurance demonstrated excellent reliability (intra-rater *ICC*: 0.87–0.91 and 0.87–0.88, respectively; inter-rater *ICC*: 0.90 and 0.89, respectively). In contrast, mouth-closing muscle strength exhibited poor intra-rater reliability (*ICC*: 0.07–0.20, *M*: 0.13) but high inter-rater reliability (*ICC*: 0.92). Mouth-closing muscle endurance demonstrated moderate to excellent reliability (intra-rater *ICC*: 0.65–0.93; inter-rater *ICC*: 0.85).

Among male subjects, mouth-opening muscle endurance was positively correlated with the AHI ($r = 0.445$, $p < 0.05$), indicating a medium effect size. Additionally, the endurance ratio of jaw-opening to jaw-closing muscles was negatively correlated with the AHI ($r = -0.469$, $p < 0.05$), also reflecting a medium effect size. These results imply that a relative imbalance favoring mouth-opening muscles may reflect or contribute to reduced airway stability. Specifically, higher mouth-opening muscle endurance relative to mouth-closing muscles was observed in males with moderate to severe OSA.

Cervical ROM, particularly in the frontal plane (lateral flexion to both sides), was significantly and strongly inversely correlated with the AHI ($r = -0.607$, $p < 0.001$). In female participants, the correlation was even stronger ($r = -0.772$, $p < 0.01$), indicating a large effect size and suggesting that reduced cervical mobility may disproportionately affect women's airway function during sleep (Fig. 2).

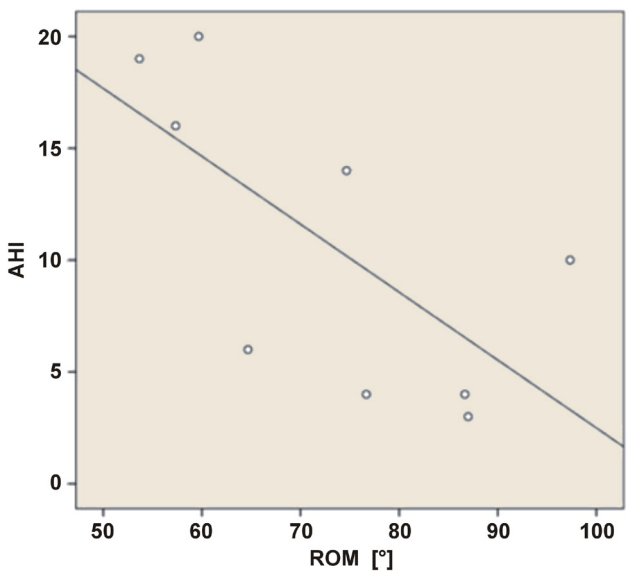

Fig. 2. Scatterplot illustrating the association between cervical range of motion (ROM) in the frontal plane and the apnea–hypopnea index (AHI) in female participants

No significant associations were identified between the AHI and neck muscle performance, head and neck posture and the FRT.

Discussion

This preliminary study explored the relationship between musculoskeletal function of the CCMC and the severity of OSA. The findings demonstrated a strong negative correlation between restricted cervical ROM, particularly lateral flexion, and OSA severity, especially in females. These findings support the hypothesis that musculoskeletal dysfunction within the CCMC may be related to OSA severity. The observed relationship may be bidirectional. Limited cervical ROM could impair compensatory head and neck movements during apneic events, while chronic overactivation of accessory respiratory muscles (e.g., scalenes) may lead to musculoskeletal adaptations and subsequent ROM restrictions. This feedback loop highlights the clinical relevance of assessing cervical function in individuals with OSA.

Contrary to our expectations, no significant correlations were found between sagittal plane ROM or forward head posture and OSA severity. These outcomes may be partially explained by the relatively low BMI of the study sample, which may reduce mechanical strain on cervical structures.

Sex-specific analyses revealed stronger correlations between limited frontal plane ROM and OSA severity in women, aligning with evidence that women experience different symptom patterns and musculoskeletal involvement in OSA.[10] These findings emphasize the importance of sex-specific diagnostic and therapeutic considerations.

Unexpectedly, in male participants, greater mouth-opening muscle endurance was associated with increased OSA severity. A possible explanation is habitual mouth opening during sleep as a compensatory response to upper airway obstruction, leading to chronic overuse and increased endurance of the mouth-opening muscles. This overuse may result in hypertrophy of the supra- and infrahyoid muscles, potentially narrowing the pharyngeal airway and contributing to increased airway resistance. These findings suggest a paradoxical adaptation that may exacerbate OSA severity. Further research is warranted to evaluate these hypotheses and clarify the underlying mechanisms.

Limitations

Several limitations should be acknowledged. First, the small sample size and absence of a control group, characteristic of preliminary studies, limit the generalizability of the findings. Second, the cross-sectional design precludes the establishment of causal relationships between CCMC function and OSA severity. Longitudinal studies are needed to determine whether interventions aimed at improving CCMC function can effectively reduce OSA severity. Additionally, mandibular ROM was not assessed, which may have limited our ability to fully characterize the functional status of the craniomandibular complex.

Conclusions

This preliminary investigation indicates that specific musculoskeletal characteristics of the CCMC are associated with OSA severity, particularly limitations in cervical lateral flexion and jaw muscle imbalances. The observed sex-specific patterns further support the importance of personalized assessment strategies. While preliminary, these findings open new directions for clinical screening and physiotherapy-based interventions.

Ethics approval and consent to participate

The study was approved by the Helsinki Committee of Soroka Medical Center, Ben-Gurion University of the Negev, Be'er-Sheva, Israel (approval No. SOR-0183-22). Written informed consent was obtained from all participants.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Written informed consent was obtained from all participants for the publication of any potentially identifiable data included in this article.

Use of AI and AI-assisted technologies

ChatGPT (OpenAI, San Francisco, USA) was used to improve the clarity and grammar of the manuscript. The authors reviewed and edited the final version and take full responsibility for the content.

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References

1. Slowik JM, Sankari A, Collen JF. *Obstructive Sleep Apnea*. Treasure Island, FL: StatPearls Publishing; 2025. https://www.ncbi.nlm.nih.gov/books/NBK459252. Accessed April 6, 2025.

2. McNicholas WT, Pevernagie D. Obstructive sleep apnea: Transition from pathophysiology to an integrative disease model. *J Sleep Res*. 2022;31(4):e13616. doi:10.1111/JSR.13616
3. Kanclerska J, Wieckiewicz M, Nowacki D, et al. Sleep architecture and vitamin D in hypertensives with obstructive sleep apnea: A polysomnographic study. *Dent Med Probl*. 2024;61(1):43–52. doi:10.17219/DMP/172243
4. Błaszczyk B, Waliszewska-Prosół M, Smardz J, Więckiewicz M, Wojakowska A, Martynowicz H. Exploring the associations of sleep bruxism and obstructive sleep apnea with migraine among patients with temporomandibular disorder: A polysomnographic study. *Headache*. 2024;65(2):242–257. doi:10.1111/HEAD.14892
5. Błaszczyk B, Martynowicz H, Więckiewicz M, et al. Prevalence of headaches and their relationship with obstructive sleep apnea (OSA) – Systematic review and meta-analysis. *Sleep Med Rev*. 2024;73:101889. doi:10.1016/j.smrv.2023.101889
6. Senaratna CV, Perret JL, Lodge CJ, et al. Prevalence of obstructive sleep apnea in the general population: A systematic review. *Sleep Med Rev*. 2017;34:70–81. doi:10.1016/J.SMRV.2016.07.002
7. Paradowska-Stolarz AM. Is malocclusion a risk factor for obstructive sleep apnea and temporomandibular disorders? An orthodontic point of view. *Dent Med Probl*. 2025;62(2):197–199. doi:10.17219/DMP/194232
8. Oliven R, Cohen G, Dotan Y, Somri M, Schwartz AR, Oliven A. Alteration in upper airway dilator muscle coactivation during sleep: Comparison of patients with obstructive sleep apnea and healthy subjects. *J Appl Physiol (1985)*. 2018;124(2):421–429. doi:10.1152/japplphysiol.01067.2016
9. Czubak JM, Stolarczyk K, Frączek M, Fułek M, Morawski K, Martynowicz H. Otolaryngological manifestations in patients with obstructive sleep apnea and continuous positive airway pressure users: A systematic review. *Adv Clin Exp Med*. 2025;34(10):1783–1791. doi:10.17219/ACEM/195243
10. Graizel-Armoni D, Greenbaum T, Brosh T, Kedem R, Emodi-Perlman A. Sex difference in the hyoid bone position in adults with obstructive sleep apnea: Systematic review and meta-analysis. *Dent Med Probl*. 2025;62(6):1177–1187. doi:10.17219/dmp/192096
11. Greenbaum T, Pitance L, Kedem R, Emodi-Perlman A. The mouth-opening muscular performance in adults with and without temporomandibular disorders: A systematic review. *J Oral Rehabil*. 2022;49(4):476–494. doi:10.1111/JOOR.13303
12. Greenbaum T, Kalichman L, Kedem R, Emodi-Perlman A. The mouth-opening muscular performance in adults and elderlies with and without dysphagia: A systematic review and meta-analysis. *Arch Gerontol Geriatr*. 2024;124:105448. doi:10.1016/j.archger.2024.105448
13. Schiffman E, Ohrbach R, Truelove E, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for clinical and research applications: Recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Group†. *J Oral Facial Pain Headache*. 2014;28(1):6–27. doi:10.11607/jop.1151
14. Rudy TE, Greco CM, Yap GA, Zaki HS, Leader JK, Boston JR. The association between research diagnostic criteria for temporomandibular disorder findings and biting force and endurance in patients with temporomandibular disorders. *Pain Med*. 2001;2(1):35–45. doi:10.1046/J.1526-4637.2001.002001035.X
15. Harris KD, Heer DM, Roy TC, Santos DM, Whitman JM, Wainner RS. Reliability of a measurement of neck flexor muscle endurance. *Phys Ther*. 2005;85(12):1349–1355. doi:10.1093/PTJ/85.12.1349
16. Muñoz-García D, Gil-Martínez A, López-López A, Lopez-De-Uralde-Villanueva I, La Touche R, Fernández-Carnero J. Chronic neck pain and cervico-craniofacial pain patients express similar levels of neck pain-related disability, pain catastrophizing, and cervical range of motion. *Pain Res Treat*. 2016;2016(1):7296032. doi:10.1155/2016/7296032
17. Williams MA, McCarthy CJ, Chorti A, Cooke MW, Gates S. A systematic review of reliability and validity studies of methods for measuring active and passive cervical range of motion. *J Manipulative Physiol Ther*. 2010;33(2):138–155. doi:10.1016/j.jmpt.2009.12.009
18. Hall TM, Robinson KW, Fujinawa O, Akasaka K, Pyne EA. Intertester reliability and diagnostic validity of the cervical flexion-rotation test. *J Manipulative Physiol Ther*. 2008;31(4):293–300. doi:10.1016/j.jmpt.2008.03.012
19. Beltran-Alacreu H, López-De-Uralde-Villanueva I, Paris-Alemany A, Angulo-Díaz-Parreño S, La Touche R. Intra-rater and inter-rater reliability of mandibular range of motion measures considering a neutral craniocervical position. *J Phys Ther Sci*. 2014;26(6):915–920. doi:10.1589/JPTS.26.915
20. Yalamanchali S, Farajian V, Hamilton C, Pott TR, Samuelson CG, Friedman M. Diagnosis of obstructive sleep apnea by peripheral arterial tonometry: Meta-analysis. *JAMA Otolaryngol Head Neck Surg*. 2013;139(12):1343–1350. doi:10.1001/JAMAOTO.2013.5338
21. Flight L, Julious SA. Practical guide to sample size calculations: An introduction. *Pharm Stat*. 2016;15(1):68–74. doi:10.1002/PST.1709

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