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Editorial

Headache phenotypes in insomnia, obstructive sleep apnea, and COMISA: Impact on diagnosis and therapy

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Headache associated with comorbid insomnia and sleep apnea (COMISA) may represent a distinct clinical phenotype. Its severity, refractoriness and unique characteristics necessitate a comprehensive, integrative therapeutic approach.

Headache and sleep disorders frequently coexist and interrelate, but are often managed in silos despite overlapping neurophysiological pathways.[1] The relationships between insomnia, obstructive sleep apnea (OSA) and primary headache disorders – particularly migraine[2] and tension-type headache (TTH)[3] – have long been observed, but remain poorly characterized. More recently, the comorbidity of insomnia and OSA, termed COMISA (comorbid insomnia and sleep apnea), has emerged as a distinct and clinically relevant phenotype,[4] yet its implications for headache diagnosis and therapy are still underexplored.[5] A recent large trial regarding COMISA and neurological comorbidities excluded headaches from the data analysis, reporting a lack of information on sleep disorders from headache clinics.[5] The International Classification of Headache Disorders, 3rd edition (ICHD-3), includes several types of sleep-related headache, but ones associated with insomnia and COMISA are not yet clearly defined as distinct headache categories.[1,6]

According to the recently revised International Classification of Sleep Disorders (ICSD-3-TR), insomnia is marked by difficulty initiating or maintaining sleep despite adequate opportunity, leading to daytime impairment.[7] It is closely associated with increased cortical excitability, sympathetic nervous system overactivation and the dysregulation of the hypothalamic–pituitary–adrenal axis.[8] These alterations heighten pain perception and are strongly implicated in central sensitization, contributing to increased vulnerability to TTH and migraine attacks.[9] Insomnia increases the risk of both TTH and migraine, the frequency of both types of headaches, as well as the disability associated with them.[10] Hyperarousal mechanisms in insomnia interfere with descending pain inhibitory pathways, often rendering headaches more resistant to pharmacological preventive treatment.[11]

In OSA, recurrent upper airway obstruction during sleep results in intermittent hypoxia, sleep fragmentation and sympathetic surges.[12] Headaches

associated with OSA tend to be mild to moderate, of the dull/pressure type, bilateral (bifrontal), and occur upon awakening.[12] Although these 'morning headaches' are generally short-lived and respond to treatment, their mechanism of action is debatable.[12] They were originally thought to be due to hypoxia-induced cerebral vasodilation and the resulting changes in intracranial pressure; however, recent studies have not found any such association.[13] In some individuals, particularly those with higher apnea–hypopnea index scores, OSA has also been associated with migraine-like presentations,[14] and even cluster headache,[15] although the mechanistic pathways have not been fully elucidated.[14]

COMISA occurs in 30–50% of OSA patients, and 30–40% of chronic insomnia patients have OSA.[16] People with COMISA exhibit greater sleep disruption, psychological distress and daytime impairment than individuals with either disorder alone. From a headache perspective, COMISA may reflect a compounded pathophysiological burden involving both hyperarousal and hypoxemia, likely increasing susceptibility to more frequent, prolonged and refractory headache episodes.[16] Headaches in COMISA may blend the characteristics of both the insomnia and OSA phenotypes, such as early morning onset with extended duration, increased severity, or the overlapping features of migraine and TTH.[17] Also, COMISA has been associated with higher rates of medication-overuse headache and psychiatric comorbidities, factors that further complicate treatment.[16]

Screening for sleep disorders should be mandatory in cases of chronic or high-frequency headaches. Validated tools, such as the Insomnia Severity Index (ISI), the STOP-Bang Questionnaire, the Epworth Sleepiness Scale (ESS), or overnight oximetry, could aid in identifying sleep-related contributors to chronic or treatment-resistant headaches. Moreover, understanding the patient's chronotype and circadian alignment can offer additional insight into headache patterns during a 24-hour period.[18]

From a therapeutic standpoint, tailored interventions are necessary. In patients with insomnia and headache, cognitive behavioral therapy for insomnia (CBT-I) has been shown to reduce the frequency of headaches and improve sleep quality.[19] In OSA-related headache, the continuous positive airway pressure (CPAP) therapy or mandibular advancement devices (MADs) can offer significant symptom relief,[17,20] with documented improvement in both headache and sleep indices. For COMISA, however, monotherapy is often inadequate. These patients may require a combination of CBT-I and CPAP, or other multimodal strategies, such as pharmacological agents that could simultaneously address sleep and pain, e.g., amitriptyline or melatonin. Behavioral strategies targeting sleep hygiene and circadian rhythms, including light therapy and sleep–wake scheduling, may also be beneficial, particularly in cases with misalignment or shift work involvement.[21]

To improve care in the COMISA population, future research should prioritize longitudinal studies exploring headache progression, neuroimaging studies of shared pathways between sleep, pain and emotional regulation, and trials testing integrated interventions in patients with comorbid sleep disorders and headache.

In conclusion, headaches associated with insomnia, OSA and COMISA represent overlapping but distinguishable clinical phenotypes that demand integrated diagnostic and therapeutic approaches. COMISA in particular should be recognized as a high-risk, underdiagnosed condition in patients with chronic or refractory headaches. Systematic screening for sleep disturbances and cross-specialty collaboration may significantly enhance outcomes, reduce pain burden and improve quality of life (QoL) in this complex clinical population.

Beyond OSA and insomnia

In addition to the well-established roles of insomnia and OSA in headache pathogenesis, a broader spectrum of sleep-related breathing disorders (SRBDs) may contribute to headache presentations and should be considered in differential diagnosis. Conditions such as upper airway resistance syndrome (UARS), central sleep apnea (CSA) and treatment-emergent CSA can also result in fragmented sleep, nocturnal hypoxia and autonomic dysregulation – each a potential driver of head pain. For instance, UARS, although subtler in its polysomnographic presentation than OSA, can induce significant sympathetic activation and micro-arousals, leading to non-restorative sleep and tension-type or migraine-like secondary headaches.[9] Similarly, central apneas may cause abrupt fluctuations in carbon dioxide levels, cerebrovascular reactivity and intracranial pressure, each capable of contributing to morning or nocturnal headaches.[13] Differentiating among these SRBDs is crucial, as their therapeutic responses vary significantly, ranging from positional therapy and expiratory pressure relief to adaptive servo-ventilation.

A growing body of research underscores the importance of considering insomnia not as a unitary condition, but as a heterogeneous group of phenotypes, some of which may coexist with SRBDs in more complex constellations. For example, paradoxical insomnia – characterized by the misperception of sleep – can overlap with milder forms of OSA or UARS, complicating the clinical picture and delaying an appropriate intervention.[22] Conversely, sleep-maintenance insomnia with frequent awakenings may reflect unrecognized respiratory arousals or limb movement disorders. In these contexts, careful use of ambulatory monitoring or full polysomnography is warranted, particularly in patients whose headache patterns correlate with disturbed or fragmented sleep.

The relevance of recognizing coexisting sleep disturbances extends beyond diagnostic clarity; it directly influences therapeutic outcomes. A patient presenting with

Table 1. Sleep disorder features potentially influencing headache presentation and management

Sleep disorder	Impact on headache	Associated mechanisms	Treatment relevance	Phenotypic headache expression	Diagnostic implications
Insomnia	increases the frequency and chronification of primary headache, especially migraine, TTH	hyperarousal, sleep fragmentation, central sensitization	CBT-I and agents targeting both sleep and headache often needed	often aggravates migraine and TTH	sleep history crucial; rule out comorbid SRBDs
OSA	may induce morning headaches; vascular instability may worsen migraine	intermittent hypoxia, sympathetic activation, vasodilation	CPAP or MAD may reduce headache severity; screen for other causes	morning headaches; a possible trigger in predisposed individuals	screen for morning headaches and other secondary causes
COMISA	dual influence – hyperarousal and hypoxia may potentiate headache burden	additive mechanisms: inflammation; impaired pain modulation	combined therapy (CBT-I + CPAP/MAD); treat both sleep disorders to improve headache control	aggravates primary headache; may complicate diagnosis and increase medication overuse	consider COMISA in refractory headache with poor sleep and mixed symptoms

The table summarizes current clinical tendencies and proposed mechanisms linking sleep disorders to headache characteristics. Rather than indicating strict phenotypes, the table outlines how insomnia, OSA and COMISA may influence headache presentation, chronification and treatment response. The data is based on clinical observations and hypotheses; further research is needed to define population-level patterns.
OSA – obstructive sleep apnea; COMISA – comorbid insomnia and sleep apnea; TTH – tension-type headache; CBT-I – cognitive behavioral therapy for insomnia; CPAP – continuous positive airway pressure; MAD – mandibular advancement device; SRBDs – sleep-related breathing disorders.

chronic migraine or TTH may show a partial response to analgesic prophylaxis or behavioral interventions if an underlying SRBD remains unaddressed.[9] Similarly, patients treated with CPAP for OSA may continue to experience headaches if comorbid insomnia or circadian misalignment is left untreated.[18] This interdependence highlights the necessity for a comprehensive sleep assessment in all patients with persistent or refractory headaches, particularly when temporal patterns suggest a sleep-related trigger.

In practice, incorporating a sleep-focused evaluation into the headache diagnostic process is warranted. Clinicians should routinely screen for symptoms such as non-restorative sleep, loud snoring, witnessed apneas, excessive daytime sleepiness, difficulty falling or staying asleep, and morning headaches. Questionnaires like the Berlin Questionnaire, STOP-Bang, ISI, and the Munich Chronotype Questionnaire (MCTQ) offer quick, validated insights. For selected patients, especially those with complex or overlapping symptoms, overnight studies are indispensable. Importantly, the use of wearable technology, including home oximetry and actigraphy, is making such evaluations more accessible and scalable.

Integrating these insights into clinical workflows not only facilitates the accurate phenotyping of sleep-related headache, but also guides rational therapeutic strategies. For instance, a patient with COMISA and mixed headache features may benefit from concurrent CBT-I and MAD, especially when CPAP adherence is poor. Another patient with CSA and chronic daily headache may respond to pharmacological agents that modulate the respiratory drive, such as acetazolamide, in conjunction with headache prophylactics. In all cases, the personalization of therapy based on the sleep and headache phenotype maximizes the likelihood of symptom resolution and QoL improvement. Table 1 summarizes the potential influences of insomnia, OSA and COMISA on headache presentation and therapeutic response, highlighting overlapping mechanisms and the need for tailored interventions.

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Editorial

AI in modern dentistry: Hype, hope, or real transformation?

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Artificial intelligence (AI) shows significant potential in supporting development and management in everyday dental practice; however, significant challenges, limitations and uncertainties remain.

Introduction

The modern world – including the field of medical sciences – is steadily advancing toward greater automation and computerization of procedures. This shift affects all aspects of clinical and laboratory workflows, from the use of three-dimensional (3D) printing in material sciences to the application of computer systems for obtaining and analyzing various types of information.[1–3] Artificial intelligence (AI) is a tool increasingly used across many fields today. First described in the 1950s, it initially referred to mechanical devices, but it soon evolved into a central focus of interest across numerous domains of modern society. Although AI continues to advance rapidly and is becoming more familiar to the general public, concerns persist regarding its potential to replace human thinking and intelligence.

The healthcare industry increasingly integrates AI, moving toward a future in which certain aspects of care may be partially driven by robots and advanced medical devices, reducing the need for direct human involvement. Through its capacity for machine learning and large-scale data analysis, AI has already begun to revolutionize many areas of medicine, including dentistry. A major focus in these fields is the transition from manual, clinician-dependent processes to more predictable, computer-assisted tools. Such technologies have potential to enhance diagnosis, treatment planning and the overall management of oral health. Since many clinical patterns are repetitive, computer systems can 'learn' to recognize similarities between conditions, which can be especially valuable in diagnosing non-specific orofacial diseases. With these learning capabilities, AI can also replicate effective treatment patterns, potentially leading to more accurate and precise procedures across all disciplines of dentistry.[4,5]

Artificial intelligence appears to offer valuable support for less experienced practitioners, enhancing their diagnostic and clinical decision-making capabilities. However, important questions remain: Is AI a reliable solution in all cases, and could it ultimately replace human intellect and clinical judgment?

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Are ethical considerations being adequately addressed, and how is data privacy ensured? As AI continues to evolve, the central debate persists – will it serve primarily as a supportive tool for clinicians, or could it eventually challenge or even substitute their roles?

Advantages of AI in dentistry

Enhancing diagnostic accuracy

The advantages of using AI in dentistry are widely discussed, particularly in relation to diagnostic accuracy and clinical efficiency. Artificial intelligence – especially systems based on deep learning – has demonstrated the ability to outperform conventional methods in detecting caries, periodontal diseases, and even more serious conditions requiring surgical intervention, such as oral cancers.

The mathematical models incorporating techniques like fractal dimension and texture analysis make it increasingly feasible to assess bone quality and the condition of the oral mucosa, using less invasive tools, such as two-dimensional (2D) radiographs and clinical photographs.[6,7] In this context, imaging data can serve as a valuable resource for identifying pathological changes and monitoring their progression.[8] For more advanced radiographic interpretation, particularly of 3D imaging modalities, convolutional neural networks (CNNs) have proven especially useful. They currently offer the highest accuracy in tasks such as tooth segmentation, classification and categorization.[9]

In addition to supporting the diagnosis of bone and oral mucosa conditions, AI can also assist with color management in restorative and prosthetic dentistry. Since human vision is not always reliable for selecting accurate shades, AI-based systems may provide more consistent and objective color-matching results.[10]

However, despite these advantages, human oversight remains essential. AI-generated recommendations should be viewed as supportive guidance rather than definitive decisions. The same principle applies to treatment planning, where AI can offer valuable insights, but cannot replace the clinician's expertise and judgment.

Treatment planning

The need for advanced treatment planning is particularly evident in surgery, prosthodontics and orthodontics. These specialties often require not only visualization tools, but also treatment simulations that can illustrate the anticipated outcomes of various procedures.

In orthodontics, for example, modern aligner systems rely heavily on AI-driven design to generate sequential tooth movements. Cephalometric analysis – an essential component of both orthodontic and orthognathic treatment planning – can also be enhanced through AI-based automated landmark identification and measurement.[11] Although AI-supported aligner planning appears highly precise, real-world clinical outcomes often deviate from the initial digital prediction. Consequently, additional refinements and mid-course corrections are frequently needed to achieve the desired final result.

Artificial intelligence can also play a significant role in planning various surgical and prosthetic procedures, including implant placement and bite reconstruction. Such planning is particularly critical in the anterior maxilla, where both functional and esthetic demands are high. Clinicians must anticipate not only the precise positioning of the implant, but also the morphology and placement of the future prosthetic crown or bridge to ensure an optimal esthetic and functional outcome.[12] AI-based tools can further assist by identifying key anatomical structures, such as the inferior alveolar canal, thereby improving the safety and accuracy of surgical procedures.[13] As previously noted, AI can also aid in the detection of oral pathologies, including cancers. The early identification facilitated by AI may significantly shorten the time between diagnosis and surgical intervention, ultimately improving patient outcomes.[4,5]

Patient communication

Artificial intelligence can be highly valuable in preparing standardized information for patients, supporting more efficient dental practice management. It can assist in creating educational content about treatment and procedures, including real-time responses to patient questions via chatbots. Natural language processing (NLP) technologies further enhance these capabilities, enabling automated appointment scheduling, check-up reminders and follow-ups – tasks that can now be handled without human intervention, with significantly improved efficiency.[14] Additionally, AI could assist in 'pre-triage' procedures, helping to prioritize patients based on the urgency of their condition.

However, a key limitation remains the lack of human oversight over the information provided, especially in response to frequently asked questions (FAQs). Despite this, the most practical current application of AI may be in reducing the clinician's time spent answering routine questions and assisting with patient anamnesis in a safe, non-intrusive way.

Critical management

Although AI offers numerous advantages, it is also associated with potential errors and inaccuracies that must be carefully considered. Only through critical oversight, with a human expert supervising its use, can AI provide reliable and unbiased information or results.

Data quality and bias

AI systems can exhibit significant bias, as their outputs are limited to the data on which they are trained. This means that important information may be overlooked, and the data presented may lack diversity, being restricted to certain ethnic groups, genders, ages, or socioeconomic backgrounds. AI models are generally not equipped to critically evaluate the limitations of their datasets, which can result in biased outputs being interpreted as definitive results.

This issue is particularly important in healthcare, including dentistry, where biased AI recommendations can affect diagnosis, treatment planning and other clinical decisions, potentially exacerbating the existing disparities. Moreover, such bias complicates the development of standardized medical protocols, as algorithmic evaluations remain far from perfect. This challenge represents a broader, unresolved problem in medicine, which may even be amplified by AI. While more advanced tools could potentially mitigate these problems in the future, significant further development of AI itself is required to achieve this goal.

Clinical integration, ethical and legal considerations

The use of AI in clinical diagnosis and procedures, particularly in surgery, carries significant implications. The reliability of AI remains uncertain, raising concerns about potential "secondary dumbness," where overreliance on AI could deskill clinicians and diminish their ability to make independent decisions. This poses a direct risk to patient safety, especially in surgical contexts, where rapid, informed action is often critical. In addition, AI systems are susceptible to bias, which increases the likelihood of errors in their outputs.

Data privacy is another key concern, as training commercial AI systems requires explicit patient consent. This includes not only personal information, but also medical images (e.g., X-rays) and laboratory results, such as blood tests.

Another critical issue is accountability in the use of AI. If an AI system provides an incorrect diagnosis or recommends an inappropriate treatment plan, who bears responsibility for the error? Artificial intelligence itself cannot be held accountable, raising complex questions about whether liability falls on the developer, the information technology (IT) specialist, the clinician, or the healthcare institution. Ethical and legal frameworks are therefore essential for the responsible implementation of AI in clinical practice.

It is equally important to emphasize that all AI-generated recommendations must be reviewed by a human professional. In this context, AI should be regarded as a supportive tool to assist clinicians, rather than as a replacement for human judgment in diagnosis and treatment.

Cost and accessibility

In addition to the limitations already discussed, the adoption of AI in dentistry may be constrained by cost considerations. Implementing AI requires standardized systems, including specialized hardware and software, which can be expensive. This financial barrier may limit adoption, particularly in smaller dental practices or in rural areas. Furthermore, some clinicians may face challenges in learning and integrating these new technologies, potentially widening the disparities among dental care providers.

These issues also raise important ethical questions regarding the equitable use of advanced AI tools in resource-limited settings, including parts of Africa and other low-income regions.

Figure 1 summarizes the key advantages and disadvantages of AI in healthcare. In the author's view, many critical questions must be addressed before AI can be fully integrated into medical practice.

Fig. 1. Use of artificial intelligence (AI) in dentistry

Future perspectives and conclusions

The future of AI, while promising, remains uncertain. Several challenges must be addressed before AI can be widely implemented in everyday medical and dental practice. Clear guidelines, as well as the rigorous validation and standardization of these tools are essential prior to their full integration. Ideally, AI should serve as a clinical adjunct rather than a replacement for human expertise.

Ethical and legal frameworks also require development to ensure safe and responsible use. While AI has potential to support clinicians, including dentists, in daily practice, it is important to remain aware of its limitations and maintain a critical perspective, particularly given that human health and well-being are at stake.

Some limitations are not inherent to the technology itself but stem from clinicians' understanding of AI. Deep learning-based algorithms, for example, can be difficult to interpret, which may complicate the justification of clinical decisions. This challenge has been highlighted by Razdan et al.,[15] underscoring the need for proper training and awareness among dental professionals.

The high expectations for AI may take time to be fully realized, if they are achievable at all. While AI holds significant potential to improve diagnostic accuracy, issues of bias and ethical transparency must be addressed. Substantial development is still required in these areas, particularly regarding clinical reliability and ethical oversight.

A key concern is whether reliance on AI could inadvertently reduce the quality of care, leading to a routine, 'mindless' approach to diagnosis and treatment. Furthermore, as diseases and treatment protocols are constantly evolving, AI systems must be capable of ongoing retraining to recognize new conditions, the emerging symptoms and the updated therapeutic approaches. Medicine teaches us that routine can be a significant risk, so strategies must be implemented to prevent overreliance on automated processes.

At present, AI cannot yet serve as a clinical standard, but it undeniably represents a critical component of the future of medicine.

In summary, AI shows considerable potential for improving development and management in everyday dental practice; however, significant challenges, limitations and uncertainties remain.

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Original paper

Prevalence of malocclusion and assessment of orthodontic treatment needs in an urban population in Poland: The SOPKARD-Junior program

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Abstract

Background. According to the World Health Organization (WHO), malocclusion is the most common oral disease in children after dental caries. Disorders pertaining to smile aesthetics and appearance can cause psychosocial problems and feelings of marginalization in children.

Objectives. The aim of the study was to assess the prevalence of malocclusion and to compare the need for orthodontic treatment in children treated with removable appliances and those who are not treated orthodontically.

Material and methods. The study sample consisted of 653 children aged 9–12 years (349 boys and 304 girls) from a selected urban population in Poland. The clinical study was based on the evaluation of Angle's classification and analysis of other occlusal characteristics (overjet, overbite, crossbite, scissor bite, crowding, diastema, and midline shift). The assessment of orthodontic treatment needs was carried out according to the Dental Health Component of the Index of Orthodontic Treatment Need (IOTN-DHC).

Results. In the sample group, 533 children (81.62%) were diagnosed with malocclusion. The most frequent diagnoses were class I malocclusions (43.80%) and class II malocclusions (35.99%), as well as crowding (37.98%). Among the children studied, 28.95% were receiving orthodontic treatment and using removable appliances. As many as half (50.26%) of the children treated with removable appliances exhibited no or minimal need for orthodontic treatment. The study revealed no significant association between the severity of patient's need for treatment and the probability of receiving orthodontic treatment with removable appliances.

Conclusions. The prevalence of malocclusion in the studied population is high. However, not all children with a diagnosed malocclusion require orthodontic treatment. For an effective plan of orthodontic care and rational budgeting, it is recommended that appropriate indicators be used to identify individuals with the most severe malocclusions who are eligible for treatment.

Keywords: children, prevalence, Index of Orthodontic Treatment Need, malocclusion

Highlights

- The prevalence of dental occlusion abnormalities in children aged 9–12 years is high.
- Despite this high prevalence, only about half of affected children require orthodontic treatment.
- Some cases do not require orthodontic treatment due to the low severity of malocclusions.
- Class I malocclusions, dental crowding and class II malocclusions are the most frequently observed.

Introduction

The results of epidemiological studies that assessed oral health in children across different age groups show that the 3 predominant conditions of the masticatory organ in children and adolescents are dental caries, malocclusion and periodontal diseases.[1–3] Due to its high prevalence, malocclusion is an important public health problem. For many individuals, malocclusion constitutes a major aesthetic issue that affects their quality of life. The orofacial region is an area of particular concern for the patient, as it attracts the most attention during interpersonal interactions and reflects the emotional state of an individual. Misaligned teeth can cause psychosocial problems related to appearance and result in marginalization or social exclusion.[4] Some malocclusions can also have a negative impact on dental and facial development, contributing to impaired oral functions (e.g., chewing, breathing, speech, swallowing). According to some authors, malocclusions increase susceptibility to dental trauma and the development of caries, and can cause periodontal problems.[5,6]

Organizing orthodontic care requires up-to-date information on the prevalence of different types of malocclusion and the need for orthodontic treatment. The data is important for the planning and subsequent implementation of preventive and therapeutic orthodontic interventions, especially in children at an early school age. On the other hand, most countries observe a constantly growing interest in orthodontic treatment not only among parents but also among adolescents themselves. This underscores the need for meticulous planning of funding and prioritization of treatment at the level of the entire population, especially in the context of public health services, where resources are limited.[7]

In Poland, reimbursement for orthodontic treatment is available for children up to the age of 12, therefore, the interest remains high. Insurance coverage is limited to treatment involving removable appliances. No indicators are used when qualifying a patient for treatment, and the application order is followed instead. Taking into consideration limited financial resources, it seems reasonable to determine whether the lack of objective guidelines, particularly in the qualification of patients for reimbursable orthodontic treatment, is appropriate.

Hence, the purpose of this study was to evaluate the prevalence of malocclusion and the existing system for qualifying patients for treatment by comparing the actual needs for orthodontic treatment in children treated with removable appliances with those who were not treated orthodontically. The analyzed data can be then used to formulate policies for healthcare systems.

Material and methods

The study was carried out within the framework of the SOPKARD-Junior program for early detection of risk factors of civilization diseases. The study received the approval from the Bioethics Committee for Scientific Research (approval No. NKBB/510-386, 395/2015). The SOPKARD-Junior is a preventive program, the main purpose of which is to assess the health status and health behavior of children and adolescents. The program welcomed all fifth-grade students from public elementary schools in Sopot, Poland. The study began after written consent had been received from children's parents or legal guardians. Information on orthodontic treatment was obtained from a questionnaire completed by the parents.

A total of 720 children were examined, but the sample group included 653 individuals, as children treated with fixed appliances and those whose parents did not complete the questionnaire regarding past orthodontic treatment were excluded.

The clinical examinations of subjects were conducted in schools, specifically in quiet classrooms without external interference, under natural or artificial illumination. The assessment of dental occlusion was carried out using latex gloves, dental mouth mirrors and millimetric rulers. The analysis did not incorporate radiographs or study casts.

The evaluation of the occlusal conditions was carried out during 1 appointment by a single dentist with 14 years of clinical experience, specializing in orthodontics.

Orthodontic variables

Molar relationship

Angle's classification was used to determine the relationship of the dental arches in the anteroposterior plane. The evaluation was based on the mutual relationship of first permanent upper and lower molars. Patients with different Angle's classification on both sides of the dental

arch were assigned to class II or class III malocclusions based on the predominant occlusal features and/or the mutual relationship of canines.[8–10]

Overjet and overbite

Overjet was classified as normal (0–4 mm), increased (>4 mm) or reverse (<0 mm). Similarly, overbite was defined as normal (0–4 mm), increased (>4 mm) or negative (<0 mm).[8–10]

Lateral crossbite and scissor bite

The analysis of the relationship of the dental arches in the transverse plane included an assessment of occlusion in the lateral segments. Lateral crossbite or scissor bite was diagnosed if it involved at least 1 tooth in the lateral segment of the arches.[2,8,9]

Midline shift

In the transverse plane, the symmetry of the dental arches in close contact was assessed based on the congruence or lack of congruence of the medial line of the upper and lower dental arches. An offset of the medial line of more than 2 mm was defined as a lack of congruence.[8,9,11]

Crowding and diastema

The presence of a diastema was determined when the distance between maxillary central incisors was more than 2 mm.[8,9]

The deficiency of space in the arch was evaluated using the index of irregularity and crowding.[12]

Orthodontic treatment need

The need for orthodontic treatment was clinically assessed according to the Dental Health Component of the Index of Orthodontic Treatment Need (IOTN-DHC) (Table 1).[13]

Statistical analysis

All statistical calculations were carried out using the data analysis software system (Statistica, v. 13.; TIBCO Software Inc., Palo Alto, USA) and a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, USA).

Table 1. Dental Health Component of the Index of Orthodontic Treatment Need (IOTN-DHC)

Grade	Description
Grade 5 (extreme treatment need)	• 5i – impeded eruption of teeth (except for third molars) due to crowding, displacement, the presence of supernumerary teeth, retained deciduous teeth, or any pathological cause • 5h – extensive hypodontia with restorative implications (more than 1 tooth per quadrant) requiring preprosthetic orthodontics • 5a – increased overjet >9 mm • 5m – reverse overjet >3.5 mm with reported masticatory and speech difficulties • 5p – defects of cleft lip and palate, other craniofacial anomalies • 5s – submerged deciduous teeth
Grade 4 (severe treatment need)	• 4h – less extensive hypodontia requiring prerestorative orthodontics or orthodontic space closure (1 tooth per quadrant) • 4a – increased overjet >6 mm but ≤9 mm • 4b – reverse overjet >3.5 mm with no masticatory or speech difficulties • 4m – reverse overjet >1 mm but <3.5 mm with recorded masticatory and speech difficulties • 4c – anterior or posterior crossbites with >2 mm of discrepancy between retruded contact position and intercuspal position • 4l – posterior lingual crossbite with no functional occlusal contact in one or both buccal segments • 4d – severe contact point displacements >4 mm • 4e – extreme lateral or anterior open bites >4 mm • 4f – increased and complete overbite with gingival or palatal trauma • 4t – partially erupted teeth, tipped and impacted against adjacent teeth • 4x – presence of supernumerary teeth
Grade 3 (moderate/borderline treatment need)	• 3a – increased overjet >3.5 mm but ≤6 mm with incompetent lips • 3b – reverse overjet >1 mm but ≤3.5 mm • 3c – anterior or posterior crossbites with >1 mm but ≤2 mm of discrepancy between retruded contact position and intercuspal position • 3d – contact point displacements >2 mm but ≤4 mm • 3e – lateral or anterior open bite >2 mm but ≤4 mm • 3f – deep overbite complete on gingival or palatal tissues without trauma
Grade 2 (mild/little treatment need)	• 2a – increased overjet >3.5 mm but ≤6 mm with competent lips • 2b – reverse overjet >0 mm but ≤1 mm • 2c – anterior or posterior crossbite with ≤1 mm of discrepancy between retruded contact position and intercuspal position • 2d – contact point displacements >1 mm but ≤2 mm • 2e – anterior or posterior open bite <1 mm but ≤2 mm • 2f – increased overbite ≥3.5 mm without gingival contact • 2g – pre- or postnormal occlusions with no other anomalies
Grade 1 (no need for treatment)	extremely minor malocclusions, including contact point displacements <1 mm

Quantitative variables were characterized by arithmetic mean (*M*) and standard deviation (*SD*). Qualitative type variables, on the other hand, were presented as means of counts and percentage values.

The Shapiro–Wilk test was used to test whether a quantitative variable came from a population with a normal distribution.

The statistical significance of the observed differences between the 2 groups was tested with Student's *t*-test. In instances where the conditions for the application of Student's *t*-test were not met or for variables measured on an ordinal scale, the Mann–Whitney *U* test was used.

Qualitative variables were analyzed with the use of the χ^2 test of independence, and the Yates' correction was applied for cell counts of less than 10. The Cochran's conditions were determined and Fisher's exact test was conducted.

In order to determine the association, strength and direction between variables, an analysis of correlation was applied by calculating Pearson's and/or Spearman's correlation coefficients. In all calculations, a *p*-value of 0.05 was considered statistically significant.

Results

The sample group consisted of 653 children aged 9–12 years, including 349 boys (53.45%) and 304 girls (46.55%). The mean age of the participants was 10.39 ±0.59 years, which was similar in both sexes. It was observed that 464 (71.06%) subjects did not receive orthodontic treatment, while 189 (28.94%) individuals were treated with removable appliances. The statistical analysis confirmed that girls were significantly more likely to undergo orthodontic treatment than boys ($p = 0.024$) (Table 2).

In the study sample, normal occlusion was present in 18.38% of the children, while abnormalities were identified in 81.62% of the subjects. Normal occlusion was more prevalent in females (23.68%) than males (13.75%) (Fig. 1).

According to Angle's classification, class I malocclusions were found in 43.80% of the subjects, with a significantly higher prevalence in males ($p = 0.038$). Class II malocclusions were identified at a comparable rate in both girls (35.19%) and boys (36.67%), whereas class III malocclusions were diagnosed in 1.88% of the subjects (Table 3).

In the study sample, an overjet within normal limits was found in 85.76% of the subjects. It occurred significantly more often in the group of girls (88.82%) ($p = 0.037$). In the female sample, a reverse overjet was not observed,

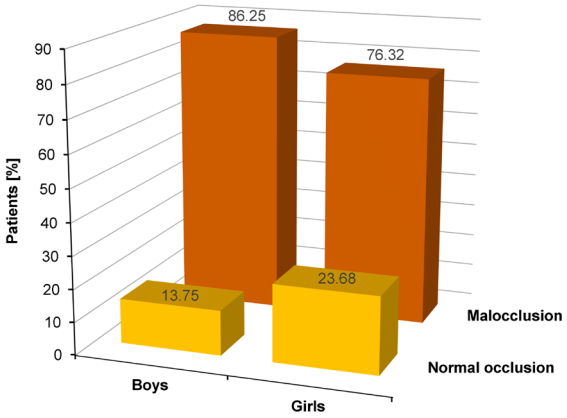

Fig. 1. Prevalence of malocclusion among study subjects based on sex

whereas it was present in 2 boys (0.58%). An increased overjet was diagnosed more often in male participants, but the difference was not statistically significant ($p = 0.058$) (Table 3).

An overbite within normal limits was present in 86.68% of the subjects. It was observed more frequently in female participants ($p = 0.002$). A negative overbite was diagnosed very rarely, affecting only 2 children. In addition, increased overbite was significantly more frequent in the male subjects ($p = 0.002$) (Table 3).

In the sample group, the occurrence of deepened overbite was evaluated in defects of class I malocclusions (8.87%) and class II malocclusions (20.85%). The results of the study proved that excessive overlapping of the upper teeth over the lower teeth more often coincides with class II defects according to Angle's classification.

Lateral crossbite was observed in 8.73% of the subjects, and scissor bite was diagnosed in 3.06% of the children. A midline shift (>2 mm) was found in 2.58% of boys and 6.58% of girls (Table 3).

Crowding of varying degrees of severity was present in 37.98% of the subjects, whereas it was absent in 37.83% of the children. In the remaining participants, the index was not applied due to the lack of erupted permanent canines or incisors. Correct tooth alignment was more frequently observed in female subjects (44.08%; $p = 0.002$) (Table 4).

Among patients who did not undergo orthodontic treatment, 52.36% of respondents exhibited no or minimal need for treatment (grades 1–2). Meanwhile, 27.37% of the children demonstrated borderline need (grade 3), and 20.25% of students exhibited definite need for treatment (grades 4–5). In the group of patients

Table 2. Characteristics of the study group

Orthodontic treatment	Girls	Boys	Total	*p*-value
No treatment	203 (66.78)	261 (74.79)	464 (71.06)	0.024*
Removable appliances	101 (33.22)	88 (25.21)	189 (28.94)	

* statistically significant ($p < 0.05$, χ^2 test). Data presented as frequency (percentage) (*n* (%)).

Table 3. Distribution of malocclusion among study subjects

Variable		Girls	Boys	Total	*p*-value
Sagittal relationship	class I malocclusion	120 (39.47)	166 (47.56)	286 (43.80)	0.038*
	class II malocclusion	107 (35.20)	128 (36.68)	235 (35.99)	0.695
	class III malocclusion	5 (1.64)	7 (2.01)	12 (1.84)	0.732
Overjet	normal (0–4 mm)	270 (88.82)	290 (83.09)	560 (85.76)	0.037*
	increased (>4 mm)	34 (11.18)	57 (16.33)	91 (13.94)	0.058
	reverse (<0 mm)	0 (0.00)	2 (0.57)	2 (0.31)	0.186
Overbite	normal (0–4 mm)	277 (91.12)	289 (82.81)	566 (86.68)	0.002*
	increased (>4 mm)	26 (8.55)	59 (16.91)	85 (13.02)	0.002*
	negative (<0 mm)	1 (0.33)	1 (0.29)	2 (0.31)	0.922
Transverse relationship	lateral crossbite	28 (9.21)	29 (8.31)	57 (8.73)	0.684
	scissor bite	10 (3.29)	10 (2.87)	20 (3.06)	0.754
	midline shift	10 (3.29)	9 (2.58)	19 (2.91)	0.590

* statistically significant ($p < 0.05$, χ^2 test). Data presented as *n* (%).

Table 4. Distribution of crowding and diastema among study subjects

Variable		Girls	Boys	Total	*p*-value
Crowding	ideal (0–1 mm)	134 (44.08)	113 (32.38)	247 (37.83)	0.002*
	mild (2–3 mm)	73 (24.01)	69 (19.77)	142 (21.75)	0.190
	moderate (4–6 mm)	35 (11.51)	48 (13.75)	83 (12.71)	0.391
	severe (7–10 mm)	13 (4.28)	9 (2.58)	22 (3.37)	0.231
	extreme (>10 mm)	0 (0.00)	1 (0.29)	1 (0.15)	0.350
Diastema		2 (0.66)	4 (1.15)	6 (0.92)	0.514

* statistically significant ($p < 0.05$, χ^2 test). Data presented as *n* (%).

treated orthodontically with removable appliances, no or minimal need for orthodontic treatment was noted in 50.26% of the subjects. A borderline need for treatment was identified in 23.28% of the children, while a definite need for treatment was found in 26.45% of the study sample (Fig. 2).

The statistical analysis did not confirm that patients with severe and extreme need for treatment are more likely to receive orthodontic treatment ($p = 0.083$).

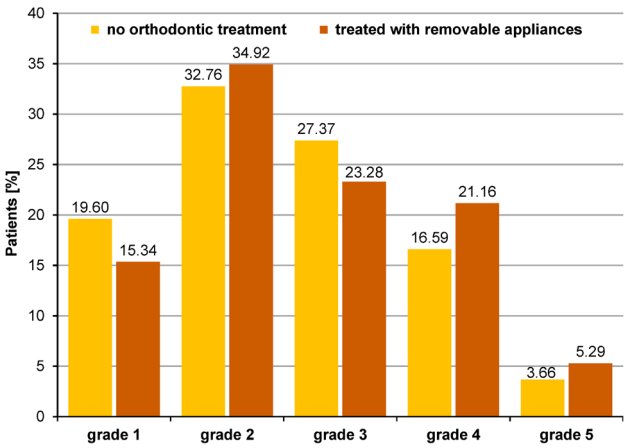

Fig. 2. Distribution of the Dental Health Component of the Index of Orthodontic Treatment Need (IOTN-DHC) in the study sample

Discussion

In Poland, the prevalence of malocclusion has been analyzed in many studies, but there are few works assessing the actual need for treatment in children. The present study demonstrated a high prevalence of malocclusion in the study population, amounting to 81.62%. A similar percentage of individuals with bite abnormalities was reported in many countries.[4,14] A higher prevalence of malocclusion (84.6–95.6%) was found in Colombia, Libya and Lithuania,[7,15,16] whereas a lower prevalence of malocclusion (56–71%) in school-aged children was reported in Brazil, Sweden, Slovenia, and Tanzania.[1,17–19]

Class I malocclusion (43.80%) was most commonly reported in the sample group. A comparison of the results of studies conducted globally yielded a similar value, as evidenced by a study from Bosnia and Herzegovina.[20] On the other hand, a higher prevalence of this abnormality, ranging from 61.4% to 72.5%, was observed in Italy, Romania, Iraq, and Morocco.[8,9,21–23] Additionally, some studies have reported a lower prevalence of class I malocclusion compared to that noted in the present study.[10]

In the analyzed study sample, class II malocclusion occurred in 35.99% of the subjects. A similar prevalence was documented in Italy.[9] The defects manifested at a higher frequency in Turkey.[10] Lower values, ranging from 9.35% to 25.40%, were reported in several countries.[7,15,21,24]

The prevalence of class III malocclusion in the sample group was the lowest, amounting to 1.88%. These abnormalities, among all malocclusions based on Angle's classification, are the least frequently diagnosed in most studies in Poland and around the world. Exceptionally different results were obtained in Mexico, where class III defects were detected in 39.09% of subjects, and they were more common than class I defects.[5]

In the present study, an increased overjet was found in 13.96% of the study subjects. These results are low when compared to those obtained by foreign authors. Some studies noted a similar number of children with an increased overjet,[9] but the values are higher in the majority of works.[4,7,8,25,26] The differences in the obtained results may be due, among other factors, to the use of different normative ranges for defining normal, increased and reverse overjet. Many studies consider a measurement range of 0–4 mm as normal,[8–10,18] a finding that aligns with the methodology employed in the present study. The upper limit of the norm adopted by other authors is 3 mm,[4,14,22] 3.5 mm[15,16] or 2–3 mm.[23]

Based on the present study, a reverse overjet was found in 0.31% of the subjects. Having analyzed the results of studies around the world, the value seems rather low. A similar or higher number of children with a reverse overjet was reported in many countries.[8,9,25] No study, however, found these values to be lower.

In the present study, an increased overlap of upper incisors on lower incisors was present in 13.02% of the subjects. Many factors affect the degree of vertical overbite. One of them is the inclination or tilting of the incisors, with the condition of the dentition playing an important role in this process. In groups with a high prevalence of caries in deciduous and permanent teeth, the incidence of increased overlap is higher. Differences may also be attributable to varying criteria for defining normal, increased and reverse overbite: 0–4 mm[8–10]; 0–3 mm[14,16]; and 1–2 mm, respectively.[23] In some works, an increased overbite is diagnosed when the upper incisors cover more than ⅔ of the surface of the lower incisors.[27]

Among the school-aged children, an increased overbite was more frequently associated with class II malocclusion, and this condition was more common among boys. Thilander et al. and Lux et al. obtained similar findings.[7,28] A higher prevalence of this abnormality was also observed in studies conducted in Colombia, Turkey, Germany, and France.[7,10,25,28] Sexual dimorphism may be related to differences in skeletal maturity and/or eruption of permanent teeth.[28] In the sample group, a negative overbite was found in 0.31% of the subjects. A similar percentage of this abnormality was documented in Italy (0.70%).[8] A higher prevalence (2.03–16.50%) was reported in numerous countries worldwide, predominantly those outside of Europe.[7,14,23] Studies have also been conducted in which no cases of open bite were reported.[29] The reason for this may be that the sample size was too small, thereby complicating the detection of less prevalent malocclusions.

A crossbite in lateral sections was present in 8.73% of the examined school-aged children. The global prevalence of this defect ranges from 5.4% to 15.2%.[8,23,28,29] In the present study, no significant differences were found in the incidence of crossbite, whether unilateral or bilateral. Although, studies conducted among children from Iraq reported a higher prevalence of this abnormality when present bilaterally.[23] Considering that the majority of the study participants were aged 10–11 years, the percentage of students with lateral crossbite may appear high. It is important to note that this disorder requires early orthodontic intervention. Many studies have identified crossbite as a crucial aspect of dental health that necessitates intervention from early childhood.[30]

A relatively rare disorder found in the transverse plane is scissor bite. In the present study, it was found in 3.06% of children. The condition is diagnosed with equal rarity worldwide.[7]

Tooth crowding is most often the result of a quantitative discrepancy between the clinical length of the dental arch and the sum of the mesial and distal widths of the teeth. Crowding was the second most prevalent (37.98%) abnormality identified in the sample group. A number of studies have documented extremely high percentages of subjects with crowding. However, these studies predominantly entailed the analysis of the amount of space on models, potentially enhancing the study's precision.[31] In the sample group, no statistically significant correlation was identified between the incidence of arch space deficiency and sex, contrary to the study by Thilander et al., who noted a higher prevalence of the condition in female subjects.[7]

Among the surveyed children from elementary schools in Sopot, 28.95% of boys and girls were treated with removable appliances. Compared to other European countries, this percentage is high. In the UK, the prevalence was 8% among 12-year-old children and 14% among adolescents aged 15–16.[32] In France, only 2.4% of children use braces, and in Latvia, 18% of boys and girls undergo orthodontic treatment.[25,33] There may be various reasons for these discrepancies, including the increasing interest in orthodontic treatment and the fact that in Poland, only removable appliances are reimbursed by the Polish National Health Fund (NFZ) for children up to the age of 12. The results of the present research demonstrate that females undergo orthodontic treatment more frequently. This finding aligns with the results of many authors.[32,34] However, there are few works that show a greater interest in braces therapy among male subjects.[35] The increased frequency of malocclusion correction needs among women may be indicative of societal stereotypes regarding gender roles, where societal norms place a higher value on physical attractiveness for women. Female patients are more likely to prioritize the aesthetic appeal of straight teeth compared to their male counterparts, which leads to a higher demand for orthodontic treatment among

females. Girls and their parents are also more likely to accept long-term orthodontic treatment.[36]

As the demand for orthodontic treatment increases, there is a growing need for reliable information regarding the actual necessity of such treatment. The diagnosis of dental occlusion abnormalities does not invariably necessitate intervention, as defects of low severity are not always an indication to start therapy. Therefore, of those reporting a desire for orthodontic treatment, it would be advisable to select individuals with the greatest treatment need. The use of dedicated indicators is instrumental in facilitating such an assessment. These tools also help classify malocclusion and prioritize qualifying patients for reimbursable orthodontic treatment.[37] One of the most commonly used indicators for assessing the need for orthodontic treatment in both children and adults is the IOTN-DHC, which was used in the present study.

The analysis showed that 23.35% of children require orthodontic treatment (grades 4 and 5). The percentage increased to 48.68% when students with grade 3 of the IOTN-DHC were also taken into account. The definite need for treatment (grades 4 and 5) is analogous to that reported in school-aged children in many countries.[8,25,38,39]

In line with the findings of most of the studies on the subject, the present study revealed no statistically significant differences with regard to the need for orthodontic treatment in relation to sex.[8,21,25,38,40] In contrast, a study conducted in Bosnia and Herzegovina identified a higher prevalence of treatment needs among female subjects.[41] The sex disparity was also noted in studies undertaken in Brazil, where a greater need for orthodontic treatment was found among male individuals.[42] In contrast, Baubiniene et al. stated that sex has an impact on the need for orthodontic treatment, but its influence changes with age.[43]

In the study population, the extreme need for treatment was found to be slightly higher among subjects treated with removable appliances compared to those who did not undergo orthodontic treatment. Although this result is expected, the statistical analysis did not confirm the significance of these differences. This finding emphasizes that children afflicted with the most severe malocclusions do not necessarily benefit from orthodontic treatment with removable braces, a treatment that is largely reimbursed in Poland.

In the group of patients treated with removable appliances, there was no or minimal need for orthodontic treatment in 50.26% of the subjects. A borderline need for treatment was found in 23.28% of the participants, while 26.45% of the children demonstrated a definite need for treatment. The absence of specific guidelines for qualifying patients for orthodontic treatment frequently results in the initiation of treatment that is not determined by the severity of malocclusion, but, for example, by the subjective assessment of the patient or the order in which the patient reports to the orthodontist. A rational solution to this situation is to establish more objective criteria for the qualification for orthodontic treatment, such as appropriate indicators.

Without objective assessment tools, judging the need for orthodontic treatment becomes subjective and unreliable. For this reason, many countries employ standardized indicators to qualify patients for free orthodontic treatment. For example, the 5-degree KIG scale is used in Germany, the IOTN in Austria, the Treatment Priority Index (TPI) in Finland, and the IOTN-DHC in the United Kingdom.[29,44,45]

The present study confirms the need for epidemiological orthodontic research, especially at this stage of occlusal development, as children represent an ideal population for planning and evaluation of the effectiveness of preventive and therapeutic programs, as well as for monitoring bite development.

Limitations

The study sample consisted of children who had not undergone orthodontic treatment or those treated with removable appliances. Children treated with fixed appliances were excluded from the study. Many researchers additionally exclude individuals with removable appliances,[3,25,30,38,43] yet this is not a universal practice.[46] According to some researchers, the exclusion of patients with any kind of appliances may create a misleading picture of the actual need for therapeutic treatment, as these individuals may still require orthodontic care.[38,47] Nevertheless, the severity of malocclusion in children treated with removable appliances may change over time, a tendency that is reflected in the study findings.

Conclusions

The prevalence of dental occlusion abnormalities in children aged 9–12 years is high, though not all cases require orthodontic treatment due to the low severity of malocclusions. The most common dental occlusion disorders are class I and class II defects, increased overjet and overbite, and dental crowding. Notably, up to half of the children currently treated with removable appliances show little or no need for such therapy. To improve the efficiency of national orthodontic care and ensure a rational allocation of resources, it is recommended that appropriate indicators be used. This approach would help prioritize treatment for individuals with more severe malocclusions who are most likely to benefit from orthodontic intervention.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee for Scientific Research (approval No. NKBB/510-386, 395/2015).

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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Original paper

Pro-inflammatory cytokines and antioxidative enzymes as salivary biomarkers of dentofacial infections in children

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Abstract

Background. Dentofacial infection resulting from untreated dental caries or periodontal disease is a serious disease that can spread to deeper tissues of the face and neck.

Objectives. The present study aimed to analyze the salivary cytokine profile and oxidative stress parameters as potential biomarkers of acute odontogenic infections in children.

Material and methods. The prospective study group (DI) consisted of 28 children aged 3–17 years with acute dentofacial infections, and the control group (CG) comprised 52 children aged 4–17 years with uncomplicated dental caries. The cytokine profile was analyzed using the Bio-Plex Pro™ Human Cytokine 27-plex kit. In addition, oxidative stress parameters, such as catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), manganese SOD (Mn-SOD), copper-zinc SOD (CuZn-SOD), total antioxidant capacity (TAC), total oxidant status (TOS), and malondialdehyde (MDA), in the saliva of children in both groups were compared.

Results. The levels of interleukin 6 (IL-6), macrophage inflammatory protein 1 alpha (MIP-1α) and tumor necrosis factor alpha (TNF-α) were significantly increased in children with dentofacial infections as compared to CG. In contrast, the levels of other pro-inflammatory cytokines, such as IL-1β, IL-1 receptor agonist (IL-Ra), IL-8, monocyte chemoattractant protein 1 (MCP-1), and MIP-1β, did not show statistically significant differences between the 2 groups. Among the measured oxidative stress and antioxidative parameters, only CAT and GR were elevated in children with dentofacial infections as compared to controls.

Conclusions. IL-6, MIP-1α, TNF-α, CAT, and GR can serve as selective biomarkers of oral cavity inflammation in children. These biomarkers can be useful in identifying and monitoring the progress and treatment of bacterial infections resulting in dentofacial inflammation.

Keywords: children, cytokines, saliva, antioxidative enzymes, dentofacial infections

Introduction

Dentofacial infections develop due to dental caries and periodontal diseases, such as gingivitis and periodontitis. They can spread to deeper tissues of the face and neck, constituting a life-threatening condition. The outcomes of dentofacial infections are related, at least in part, to the type of bacterial infection, host immunity, dietary factors, and the oral hygiene status.[1–5] Indeed, host immune responses, the quantity and virulence of bacteria, as well as the disease status all play critical roles in the development of bacterial infections, and determine prediction, prevention and intervention with regard to the infection. *Streptococcus mutans*, *Actinomyces* spp. and lactobacilli are considered the main bacteria responsible for the development of dentofacial infections,[6] as they produce a biofilm covering tooth surfaces and gum pockets.

While immune responses in cavities are triggered when odontoblasts in dental pulp become inflamed, the role of saliva proteins in this inflammation process is not fully understood. Therefore, understanding the innate markers underlying dentofacial infections is crucial to ensure oral health and effective protection against the development of cavities and their consequences, such as periodontal tissue inflammation. This focus is consistent with the recent strong emphasis on the natural defense system of the oral cavity and the role of saliva.[7,8]

Unlike blood, saliva is obtained noninvasively, which matters, especially in the case of children. Only a small amount of saliva is needed to analyze a full panel of cytokines and chemokines.[2,3,9,10] Saliva contains various proteins, such as cytokines, chemokines, proline-rich glycoproteins, mucins, immunoglobulins, agglutinins, lactoferrin, cystatins, and lysozyme,[11] which are important in the development of inflammatory processes and their prevention. The role of inflammatory processes in dentofacial infections in children and the contribution of saliva are not fully understood.

Among the cytokines present in saliva, interleukin 1 beta (IL-1β), IL-2, IL-6, IL-8, tumor necrosis factor alpha (TNF-α), and interferon gamma (IFN-γ) play a key role in stimulating the immune system to fight off infection and inflammation. For example, IL-1β plays a role in inflammatory responses, cell death, apoptosis, and bone resorption. It is particularly involved in periodontal diseases, and is linked to TNF-α and IL-6.[12] Chemokines, such as IL-8, play a key role in the activation and migration of neutrophils, the first line of defense against bacteria that enter periodontal tissues from the circulatory blood.[13,14] When neutrophils reach the infected tissues through chemotaxis, they phagocytize and destroy the microorganisms by producing reactive oxygen species (ROS) and proteolytic enzymes. On the other hand, anti-inflammatory cytokines, such as IL-1 receptor antagonist (IL-1Ra) and IL-10, inhibit the production of pro-inflammatory cytokines and help to reduce inflammation.[15] In addition, cytokines such as IL-6 may have both pro-inflammatory and anti-inflammatory properties.

One key aspect related to saliva biology is the relationship between oral infection and inflammation and the role of oxidative stress in these processes. Oxidative stress refers to an imbalance between the production of ROS and the cellular antioxidant defense mechanisms. Reactive oxygen species are highly reactive molecules that can cause damage to cellular components and lead to lipid peroxidation, resulting in the generation of malondialdehyde (MDA).[16] If not countered by antioxidants, oxidative stress can lead to cellular damage and contribute to the development of various diseases. Among the markers of oxidative stress that can be measured in saliva, catalase (CAT) is an enzyme that catalyzes the breakdown of hydrogen peroxide (H_2O_2) to water (H_2O) and oxygen (O_2).[17–19] Glutathione reductase (GR) catalyzes the reduction of glutathione disulfide (GSSG) to its reduced form, glutathione (GSH), being an essential component of the glutathione antioxidant system.[20,21] Total antioxidant capacity (TAC) is a measure of the overall antioxidant capacity,[22] and total oxidative stress (TOS) is a measure of the overall oxidative stress level in biological samples.[19] Superoxide dismutase (SOD) and its isoforms, manganese SOD (Mn-SOD) and copper-zinc SOD (CuZn-SOD), catalyze the conversion of superoxide anions (O_2^-) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2).[23]

A relationship between dental infections and cellulitis and oxidative stress markers in saliva has recently been suggested.[24,25]

Hence, there is a pressing need for further research focusing on the role of saliva in inflammatory responses to dentofacial infections in children. By elucidating the molecular mechanisms underlying these processes, future studies have the potential to uncover novel diagnostic and therapeutic strategies for improving the management of these infections, and for promoting oral health in pediatric populations.[7,8,14,26]

Our study fills a critical gap in the literature by examining the role of salivary biomarkers in pediatric dentofacial infections. By offering novel insights into the pathogenesis and management of these conditions, we aim to advance both scientific understanding and clinical practice in pediatric oral healthcare.

Therefore, the present work aimed to evaluate whether salivary cytokines and oxidative stress parameters may serve as biomarkers of acute odontogenic oral and facial infections in children.

Material and methods

Study groups

The study was conducted in the years 2020–2022 in the Clinic of Pediatric Otolaryngology, Head and Neck

Surgery of the Department of Pediatric Surgery at the Medical University of Silesia (SUM), Katowice, Poland. It aimed to investigate the prevalence and potential biomarkers of acute dentofacial inflammation in children. The research embraced 2 groups of patients: a study group (DI) of 28 children (7 girls and 21 boys, aged 3–17 years; mean age: 8.67 ±4.64 years) with acute dentofacial infections; and a control group (CG) of 52 children (16 girls and 36 boys, aged 4–17 years; mean age: 8.38 ±3.67 years) with uncomplicated dental caries. The diagnosis of dental-related inflammatory conditions was determined according to the criteria established by the World Health Organization (WHO), which include clinical, radiographic and laboratory factors used to diagnose and classify different types of oral and dental diseases.[27] The WHO criteria provide a standardized approach for diagnosis, which can aid in developing treatment plans and tracking the disease over time. The study was approved by the Bioethical Committee of the Medical University of Silesia (SUM), with reference number PCN/0022/KB1/1/20.

The inclusion criteria were children with dentofacial infections who were free of any systemic diseases and had not taken any medications in the past month. The exclusion criteria comprised the occurrence of systemic conditions that prevented the continuation of the study, the lack of cooperation of a child and the refusal of a parent to participate in the study. All legal guardians and children over the age of 16 signed an informed consent form for the study (Fig. 1).

The examinations were conducted by a single doctor (B.E.O.W.), visually and by touch, and then intraorally using a probe and a mirror. In the DI group, the number of teeth with caries and teeth causing inflammation was determined. In CG, the number of teeth with uncomplicated caries was determined. Saliva was collected in the morning between 8 a.m. and 11 a.m. on an empty stomach, after rinsing the mouth with water and waiting for 10 min. The saliva was then centrifuged at 3,000 rpm for 10 min at 4°C in a Centurion centrifuge (Centurion Scientific Ltd., Chichester, UK) and stored at –80°C for further studies. The importance of the procedure was explained to parents and older children.

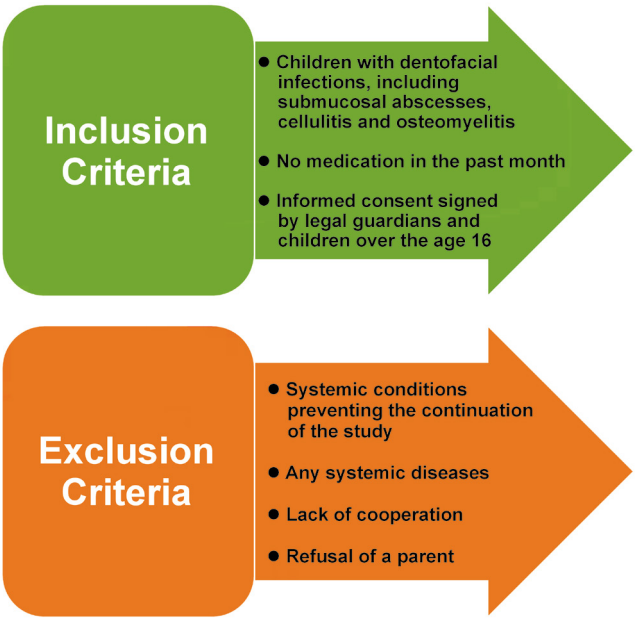

Fig. 1. Inclusion and exclusion criteria

Assessment of inflammatory mediators

The cytokine and chemokine levels were assessed using the Bio-Plex® 200 System and the Bio-Plex Pro™ Human Cytokine 27-plex kit (Bio-Rad Laboratories, Hercules, USA), according to the manufacturer's instructions (Fig. 2A).[28–30] The analyses were performed in the Department of Microbiology and Immunology, Faculty of Medical Sciences in Zabrze, Medical University of Silesia (SUM), Katowice, Poland. All procedures followed the Good Laboratory Practice (GLP) standards. To avoid bias, all samples were anonymized and numbered. All analytical methods were under continuous interlaboratory quality control, and met the criteria of the Central Center for Quality Testing in Laboratory Diagnostics (Lodz, Poland) and Labquality (Helsinki, Finland).

Assessment of oxidative stress and antioxidative potential

The activity of SOD and its isoenzymes (Mn-SOD and CuZn-SOD) was measured as described by Oyanagui.[31] In this method, xanthine oxidase produces superoxide anions that react with hydroxylamine, forming nitric ions. These ions react with naphthalene diamine and sulfanilic acid, generating a colored product, which is proportional to the amount of superoxide anions produced and negatively proportional to the activity of SOD. Absorbance was measured at a wavelength of 550 nm. The enzymatic activity of SOD was expressed in nitric units (NUs). The assessment of Mn-SOD and CuZn-SOD activity employed similar approaches, using potassium cyanide (KCN) as the inhibitor of CuZn-SOD activity. The activity of SOD is equal to 1 NU when it inhibits nitric ion production by 50%. The activity of SOD, Mn-SOD and CuZn-SOD was expressed in NU/mg of protein.

CAT activity was evaluated according to the method described by Johansson and Borg.[17] The method is based on the reaction of the enzyme with methanol in the presence of optimal concentrations of H_2O_2. Formaldehyde produced in the reaction is measured spectrophotometrically at 550 nm as the Purpald® dye (Avantor Performance Materials Poland, Gliwice, Poland). GR activity was measured as described by Richterich and Colombo.[32] The activity was expressed in IU (international unit)/g of protein.

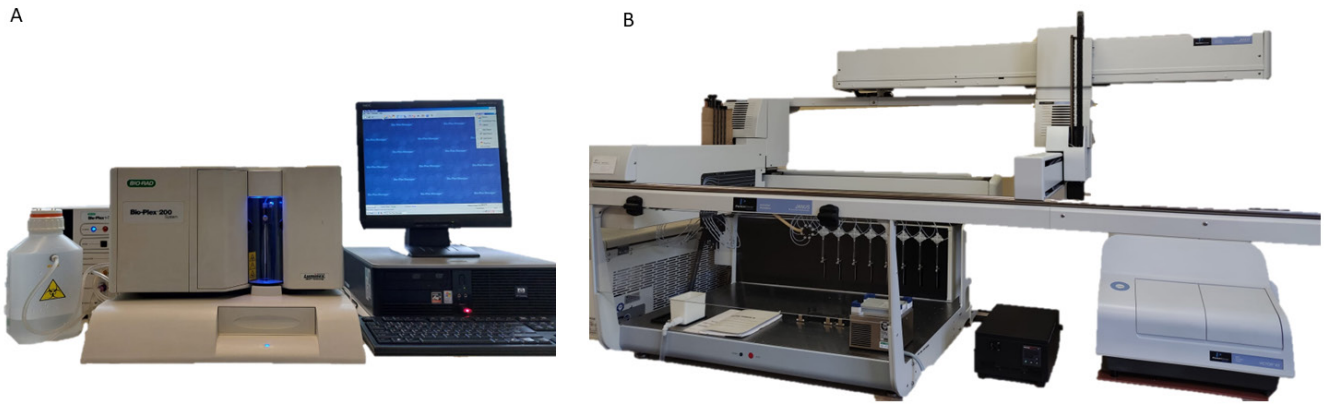

Fig. 2. Bio-Plex 200 System (A) and the JANUS automated analyzer (B)

TAC and TOS were measured according to Erel's protocols.[19] When assessing TAC, a colored 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{*+}$) solution is decolorized by the antioxidants present in the analyzed sample. The reaction efficiency depends on the level of antioxidant compounds. The color change was measured as a change in absorbance at 660 nm, using an automated analyzer (JANUS™; PerkinElmer Inc., Waltham, USA) calibrated with Trolox® (Sigma Aldrich Chemie, Steinheim, Germany) (Fig. 2B). The data is shown in mmol/g. The TOS assay is based on the oxidation of ferrous ions to ferric ions by the oxidant species present in an acidic medium. The measurement of ferric ions with xylenol orange was analyzed as a change in absorbance at 560 nm, using the same automated analyzer calibrated with H_2O_2. The data is expressed in µmol/g.

The MDA levels as a marker of lipid peroxidation were measured fluorometrically as 2-thiobarbituric acid-reactive substances (TBARS), as described by Ohkawa et al.,[33] at 515 nm and 522 nm excitation wavelengths, using the automated analyzer. The TBARS values are expressed as MDA equivalents. Tetraethoxypropane was used as the standard. The concentrations are given in µmol/g.

Statistical analysis

Statistical analysis was performed using Statistica 13 (TIBCO Software Inc., Palo Alto, USA). Student's *t* test and the Wilcoxon signed-rank test were used for the statistical analysis of parametric and nonparametric samples, respectively.

The study sample size was calculated based on mean and standard deviation ($M \pm SD$), as exhibited in the paper by Menon et al.[34] The accepted level of significance was set at $p \leq 0.05$, with a wanted power of 90%. Using a sample size of 24 patients per group, the study would have had a power of 90.9% to yield statistically significant results under the abovementioned conditions.

Results

Impact of dentofacial infection on the levels of pro-inflammatory and anti-inflammatory cytokines in saliva

Table 1 shows the levels [pg/mL] of all cytokines detected in the saliva of all the patients examined. The table includes the $M \pm SD$, minimum (min) and maximum (max) values for each cytokine in each group, as well as the *p*-values indicating the level of statistical significance of the differences between the 2 groups. The results indicate that among the measured pro-inflammatory cytokines, the levels of IL-6 (Fig. 3A), macrophage inflammatory protein 1 alpha (MIP-1α) (Fig. 3B) and TNF-α (Fig. 3C) were significantly higher in children with dentofacial infections as compared to controls with uncomplicated dental caries. In contrast, the levels of IL-1β, IL-1 receptor agonist (IL-Ra), IL-8, monocyte chemoattractant protein 1 (MCP-1), and MIP-1β did not show statistically significant differences between the 2 groups.

Impact of dentofacial infection on oxidative stress and antioxidative parameters in saliva

Table 2 presents the results for oxidative stress and antioxidative factors in the saliva of children with dentofacial infections as compared to controls. The table includes the $M \pm SD$, min and max values, and the *p*-values for each parameter in both groups. Among the studied oxidative stress and antioxidative indicators, the activity of CAT and GR was higher in the DI group as compared to CG, indicating greater antioxidative protection. Specifically, the mean CAT level was 25.52 ±14.50 IU/g, with a minimum of 6.04 IU/g and a maximum of 77.05 IU/g in controls. In children with dentofacial infections, the mean CAT level was higher by 68%, at 42.99 ±19.97 IU/g, with a minimum of 15.63 IU/g and a maximum of 95.42 IU/g (Fig. 4A).

Table 1. Levels of pro-inflammatory and anti-inflammatory cytokines and chemokines [pg/mL] in the saliva of children with dentofacial infections as compared to controls

Variable	Groups						*p*-value
	DI			CG			
	M ±*SD*	min	max	*M* ±*SD*	min	max	
IL-1β	303.18 ±304.54	0.07	1,141.27	167.31 ±181.08	16.22	931.73	0.060
IL-Ra	13,023.07 ±14,951.81	351.39	55,289.18	21,925.36 ±35,614.43	785.40	151,235.55	0.710
IL-6	132.42 ±220.74	0.56	916.60	24.54 ±49.00	0.74	328.94	0.000*
IL-8	956.69 ±1,582.35	0.27	8,168.46	691.86 ±884.51	13.85	3,888.11	0.720
MCP-1 (MCAF)	38.45 ±30.29	0.11	124.16	41.83 ±48.46	2.89	259.23	0.450
MIP-1α	14.86 ±22.02	0.56	93.73	4.61 ±4.36	1.13	26.38	0.020*
MIP-1β	35.29 ±50.36	0.49	180.63	16.21 ±17.76	2.63	103.18	0.100
TNF-α	39.61 ±33.14	3.23	121.91	24.93 ±18.84	5.61	93.38	0.040*

Groups: DI – children with acute dentofacial infections; CG – control group (children with uncomplicated dental caries).
IL – interleukin; IL-Ra – IL-1 receptor agonist; MCP-1 (MCAF) – monocyte chemoattractant protein 1 (monocyte chemotactic and activating factor); MIP – macrophage inflammatory protein; TNF-α – tumor necrosis factor alpha; *M* – mean; *SD* – standard deviation; min – minimum; max – maximum; * statistically significant.

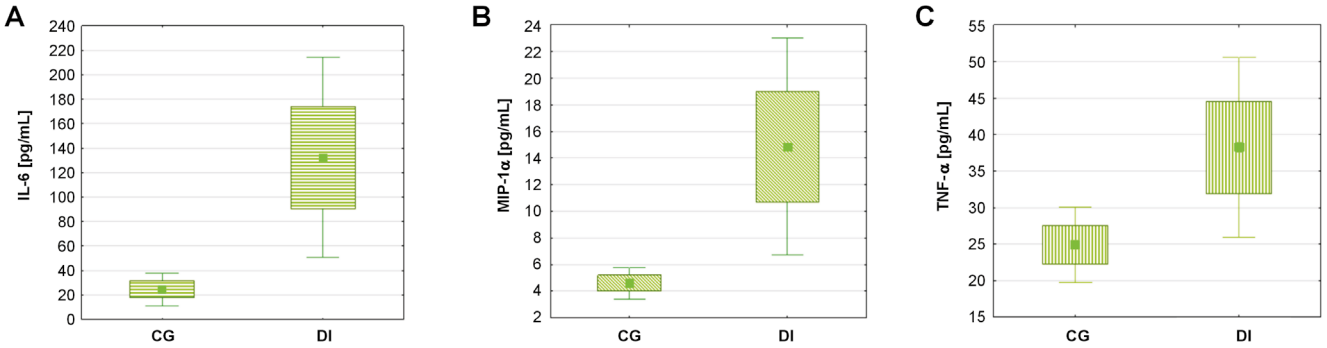

Fig. 3. Box–whisker plot showing the values for the interleukin 6 (IL-6) (A), macrophage inflammatory protein 1 alpha (MIP-1α) (B) and tumor necrosis factor alpha (TNF-α) (C) levels in the dentofacial infections (DI) group and the control group (CG)

The box represents interquartile range (*IQR*), the square inside the box is median (*Me*), and the whiskers represent the min and max values. The dots outside the whiskers represent outliers ($p < 0.01$).

Table 2. Levels of various oxidative stress parameters and antioxidative factors in children with dentofacial infections as compared to controls

Variable	Groups						*p*-value
	DI			CG			
	M ±*SD*	min	max	*M* ±*SD*	min	max	
CAT [IU/g]	42.99 ±19.97	15.63	95.42	25.52 ±14.50	6.04	77.05	<0.010*
GR [IU/g]	6.94 ±5.99	0.43	26.36	3.16 ±2.73	–0.02	15.32	<0.010*
TAC [mmol/g]]	0.05 ±0.11	0.00	0.49	0.08 ±0.20	0.00	1.24	0.850
TOS [μmol/g]	1.98 ±2.42	0.04	10.13	2.00 ±1.89	0.20	8.15	0.600
SOD [NU/mg]	5.59 ±3.13	1.54	10.56	5.92 ±4.59	0.62	22.36	0.960
Mn-SOD [NU/mg]	13.49 ±32.24	0.37	105.00	3.45 ±3.30	0.15	13.86	0.450
CuZn-SOD [NU/mg]	1.68 ±1.75	0.00	6.00	2.53 ±2.42	0.00	9.57	0.450
MDA [μmol/g]	0.26 ±0.29	0.03	0.98	0.23 ±1.17	0.01	1.17	0.800

Groups: DI – children with acute dentofacial infections; CG – control group (children with uncomplicated dental caries).
CAT – catalase; GR – glutathione reductase; TAC – total antioxidant capacity; TOS – total oxidative stress; SOD – superoxide dismutase; Mn-SOD – manganese SOD; CuZn-SOD – copper-zinc SOD; MDA – malondialdehyde; * statistically significant.

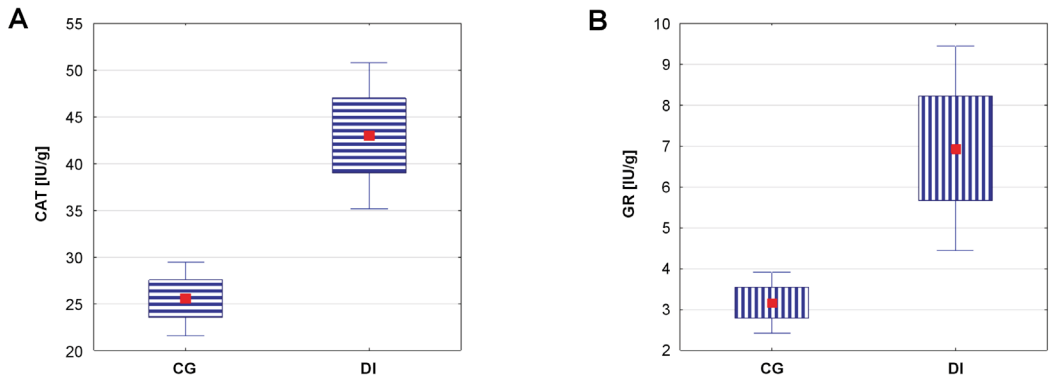

Fig. 4. Box–whisker plot showing the values for the catalase (CAT) (A) and glutathione reductase (GR) (B) levels in the dentofacial infections (DI) group and the control group (CG)

The box represents *IQR*, the square inside the box is *Me*, and the whiskers represent the min and max values. The dots outside the whiskers represent outliers ($p < 0.01$).

Regarding GR, the mean activity in CG was 3.16 ±2.73 IU/g, with a minimum of –0.02 IU/g and a maximum of 15.32 IU/g. In the DI group, the mean activity was higher by more than 100%, at 6.94 ±5.99 IU/g, with a minimum of 0.43 IU/g and a maximum of 26.36 IU/g (Fig. 4B). The differences between the 2 groups in terms of all other oxidative stress parameters and antioxidative factors did not reach statistical significance.

Discussion

This study aimed to determine the levels of interleukins and oxidative stress parameters in the saliva of children with dentofacial infections. This is an important clinical problem, as inflammation in the oral cavity and face region can be caused by several common dental conditions, such as tooth infections, abscesses and periodontal diseases. To date, there have only been a limited number of studies on this topic in the literature, making this research a pioneering effort in the field. To address inflammation in dentistry, strategies such as utilizing chitosan coatings, exploring other natural polymers and embracing the principles of green dentistry can aid in mitigating inflammatory responses and promoting oral health.[35–37] Caries was excluded from this study to focus specifically on dentofacial infections and their associated biomarkers. Nevertheless, there is a need to establish certain biomarkers of dentofacial infection as important prognostic and preventive factors for caries and the threatening complications of this disease.[27] The results of the present study indicate that saliva can be used to study biomarkers that may impact the development of acute deep carious infections in children.

The results of our study indicate that the detection of IL-6 and TNF-α in saliva samples can be used as an indicator of acute inflammation within the oral cavity in children. Indeed, both cytokines were significantly elevated in children with dentofacial infections as compared to controls. They are key mediators of acute inflammation and are responsible for specific immune responses during inflammation. Our results are in line with the reported literature. For example, Gornowicz et al. in their study investigated the levels of TNF-α, IL-6 and IL-8 in patients with and without dental caries, and found statistically significantly elevated levels of these cytokines in the saliva of patients with caries.[13] Moreover, Menon et al. showed that IL-6 significantly correlated with early enamel caries (EEC), and its levels decreased after caries treatment in children.[34] Sharma et al. studied the same cytokines in children with EEC and came to similar conclusions.[38] Zielińska et al. showed that higher levels of TNF-α correlated with high levels of aerobic bacteria, indicating an early immune response.[10] Rinderknecht et al. showed an elevated level of pro-inflammatory interleukins IL-6 and IL-8 in children with periodontitis, and proposed that they might serve as a prognostic or confirming factor for oral inflammation.[11] In contrast, Yoshida et al. showed a significant decrease in the levels of TNF-α, IL-1β, IL-6, and IL-8 under the influence of periodontitis treatment in children with gum inflammation and cerebral palsy.[15] Overall, the results of the present study and literature data suggest that TNF-α and IL-6 may serve as reliable biomarkers of caries and oral inflammation in children. TNF-α, a pivotal cytokine in immune responses, may play a role in dentofacial anomalies, particularly in periodontal diseases, although the exact impact remains under investigation due to inconsistent findings across studies.[39]

Our study indicates that MIP-1α can also serve as a biomarker of dentofacial infection.

Several oxidative stress parameters have been proposed in the literature as potential biomarkers of acute oral and facial inflammation. One of them is the MDA level as a marker of lipid peroxidation, since inflammation can lead to enhanced oxidation of lipids in cell membranes. In our study, the MDA levels remained unchanged in children with dentofacial infections, suggesting that the levels of oxidative stress did not reach the threshold required for an increase in this parameter. The lack of changes in TOS in the DI group as compared to controls confirms this notion.

It is noteworthy that matrix metalloproteinase 8 (MMP-8) and MMP-20 may serve as additional potential biomarkers for assessing the severity of early childhood caries (ECC) and for monitoring treatment outcomes in pediatric patients.[26]

The novel results of the present study indicate increased activity of CAT and GR in the saliva of children with dentofacial infections as compared to CG. These effects may be responsible for the lack of changes in the MDA levels in these children, as both enzymes exert potent antioxidative protection. Indeed, changes in GR activity and/or expression levels have been reported in various diseases, including inflammatory conditions. Glutathione is an important antioxidant that helps protect cells from oxidative damage. Changes in the glutathione levels have been frequently assessed as an indicator of oxidative stress and inflammation in oral and facial tissues. It is important to note that these parameters may be influenced by other factors, such as diet, lifestyle and the disease state.[40,41] Surprisingly, we did not observe any alterations in the activity of SOD and its isoenzymes, Mn-SOD and CuZn-SOD, which have also been used as indicators of oxidative stress in oral and facial tissues.[17,31]

While the results of the present study on saliva-related inflammatory biomarkers are novel and highly promising, it should be noted that collecting saliva samples from young children can be challenging. Some children are uncooperative and do not want to spit into the container, making it difficult to obtain a sufficient sample. Additionally, children who are being prepared for general anesthesia are usually fasting and poorly hydrated, which results in a very poor saliva flow and makes it difficult to collect even a small amount of saliva. Thus, our study also highlights the importance of developing more convenient and noninvasive methods for collecting saliva samples from children.

Limitations

In addition to its numerous advantages, it is important to acknowledge the limitations of saliva-based diagnostics. While saliva offers a noninvasive means of sample collection, its composition can be influenced by various factors, such as diet, the hydration status, the circadian rhythm, and medications, which may introduce variability in the biomarker levels and affect the accuracy of diagnostic tests. Furthermore, the sensitivity and specificity of saliva-based assays may vary depending on the target biomarker and the detection method employed, necessitating validation studies to ensure reliability and reproducibility. Additionally, the current understanding of salivary biomarkers and their diagnostic utility for specific oral healthcare problems is still evolving, requiring further research to establish standardized protocols and reference ranges.

Conclusions

This study suggests that the levels of selected pro-inflammatory cytokines, such as IL-6, MIP-1α and TNF-α, and the activity of antioxidative enzymes, such as CAT and GR, can be used as biomarkers of inflammatory states of the oral cavity and face in children. These biomarkers can provide an insight into inflammatory and oxidative stress responses in children, and may aid in understanding the underlying mechanisms of the disease and in developing potential therapeutic strategies.

Ethics approval and consent to participate

The study was approved by the Bioethical Committee of the Medical University of Silesia (SUM), Katowice, Poland, with reference number PCN/0022/KB1/1/20. All legal guardians and children over the age of 16 signed an informed consent form for the study.

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.

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Original paper

Self-assessment skills of undergraduate students in operative dentistry: Preclinical performance and gender

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Abstract

Background. Self-assessment is key to improving the quality of work performed by dental professionals. The ability to accurately self-assess has been found to correlate with better clinical performance, making it an important skill for students to master during their dental education. Furthermore, studies conducted in dental schools across the world have shown that lower-performing students tend to overestimate their abilities compared to their peers.

Objectives. This study aimed to evaluate the self-assessment skills of dental students in pre-clinical operative dentistry and to investigate the impact of gender on these skills.

Material and methods. Third-year undergraduate dental students ($N = 335$) took 2 pre-clinical practical exams: class II composite preparation and restoration. Students self-assessed each assignment using a standardized rubric, and 6 calibrated faculty members graded all procedures blindly and independently. The difference between students' self-assessment scores (S) and mean faculty grades (F) reflected the students' self-assessment skills and was referred to as the Student-Faculty (S-F) gap. A positive S-F gap indicates that students overestimate their work, while a negative S-F gap indicates that students underestimate their work. Data was stratified by gender and by faculty-determined student performance, and then statistically analyzed.

Results. The study demonstrated a statistically significant difference between faculty grades and students' self-assessment scores. Positive S-F gaps were observed across all procedures, indicating overestimation, with a mean S-F gap of 11.4 ±9.9%. A negative correlation was found between faculty grades and S-F gaps. Higher-performing students showed smaller S-F gaps (4.8 ±5.3%) compared to lower-performing students (21.2 ±9.68%). Furthermore, male students showed significantly higher S-F gaps (14.0 ±10.3%) compared to females (9.7 ±9.4%).

Conclusions. Overestimation was more prevalent among lower-performing and male students compared to higher-performing and female students. Future investigations should consider exploring effective interventions and educational strategies aimed at improving students' self-awareness and their ability to accurately assess their performance.

Keywords: dental students, self-assessment, educational measurement, operative dentistry

Introduction

The ability to self-assess is crucial for engaging students in the active learning process and strengthening their sense of responsibility in achieving the desired learning outcome. This is of particular importance in dental school, where students need to learn specific clinical skills and work on improving their technical abilities. Notably, in the USA, the Commission on Dental Accreditation (CODA) places a high value on a student's ability to be responsible for their own learning by including self-assessments among the standards required in the dental predoctoral curriculum.[1] Interestingly, previous studies have demonstrated that lower-performing students tend to overestimate their abilities, whereas higher-performing students are better at accurate self-assessment.[2–5]

The adoption of self-assessment skills is needed in current dental education to encourage active learning instead of passive learning with a uni-directional flow of information from the faculty to the student.[6] This can lead to targeted learning and a personalized improvement plan, resulting in better pre-clinical performance. Self-assessment skills have been found to improve with clinical experience, as evidenced by fourth-year students' scores correlating much more closely with faculty scores compared to first-year students.[7] Additionally, the ability of a student to improve their self-assessment skills has been found to lead to improved performance.[8] Given the necessity of acquiring this skill and the benefits that it provides for predoctoral education, it is crucial that dental students spend sufficient time improving their ability to self-assess. The groundwork for these studies was laid by Kruger and Dunning in 1999. Their work demonstrated that low performers tend to have poor self-evaluation skills but also highlighted that improving skills in a particular domain enables better analysis of one's abilities.[9]

The coronavirus disease 2019 (COVID-19) pandemic increased the significance of student self-assessment due to the mandatory transition to virtual education.[10–13] Consequently, innovative approaches such as Blended Intensive Programs (BIPs) have emerged.[14] These programs integrate online learning with brief in-person instruction sessions.[15] The shift to distance education has underscored the significance of self-directed and self-assessed learning.[16]

Numerous studies have been conducted on dental student self-assessments, both in the USA[17–20] and globally.[21,22] One international study compared dental student self-assessment abilities in the USA and Japan.[22] Although different pre-clinical exercises were performed, the researchers found that both countries had lower-performing students overestimating their performance and higher-performing students underestimating their performance.[22] However, there was a larger range between lower- and higher-performing students in the USA, while Japanese dental students self-assessed themselves more similarly to their faculty graders.[22] One potential explanation for the differences between the 2 countries is the relative cultural homogeneity of students and faculty in Japan compared to those in the USA, who tend to come from a multitude of backgrounds.[22] In this study, most students at the Faculty of Dentistry of Ain-Shams University, Cairo, Egypt, are Egyptians, with a small percentage of international students. The faculty members, on the other hand, are all Egyptians and are all graduates of the same dental school.

Similar studies have been conducted in Syria,[23] Turkey[2] and Saudi Arabia.[24] They found discrepancies in the self-assessment abilities between lower- and higher-performing students. The studies suggest that more training and practice can improve self-assessment skills among dental students.[2,23,24] Additionally, a multicenter study in Germany found that student self-assessments serve as a useful indicator of deficiencies in required competencies among undergraduate dental students.[25]

To our knowledge, this is the first study evaluating dental students' self-assessment skills and performance in Egypt, with a large sample size of over 300 students from the same class and multiple (6) faculty graders.

The goal of this study was to evaluate dental students' self-assessment abilities in relation to their coursework in operative dentistry. The null hypothesis for this study posited that there would be no difference between the self-assigned scores and those given by faculty.

It is also important to evaluate the influence of gender on student self-assessment because performance pressure is a significant stressor for dental students, especially female students.[26,27] Previous studies have revealed that male students tend to overestimate their performance compared to female students in pre-clinical restorative coursework.[24,28] Studies evaluating self-assessment among medical students have found that females have lower confidence in their abilities and underestimate their performance more than their male counterparts.[29–31] This study is the first in Egypt to examine the impact of gender on self-assessment among predoctoral dental students. The null hypothesis was that there would be no difference in self-assessment abilities between male and female students.

Material and methods

The study was approved by the ethics committee of the Faculty of Dentistry of Ain-Shams University, Cairo, Egypt (IRB approval No. FDASU-Rec IR092206). The participants were third-year students enrolled in the five-year Bachelor of Dental Surgery program at the Faculty of Dentistry of Ain-Shams University. The students participated in the pre-clinical operative dentistry course for the academic year 2021–2022. Six full-time faculty members from the Department of Operative Dentistry, with 10–30 years of experience, taught the entire course and graded the students' performance.

As part of the course requirements, students completed pre-clinical formative assessments with guidance from the Department of Operative Dentistry. They performed various cavity preparations and restorations on acrylic typodonts, ranging from Class I to V. At the end of the course, students took final practical competency examinations without any guidance or assistance from the faculty. The students were evaluated on their performance in 2 specific pre-clinical procedures: Class II resin composite cavity preparation and restoration.

At the beginning of the course, the course director provided a review of the criteria rubrics to ensure that students had a clear understanding of the criteria for each procedure. The rubrics included a detailed itemization of the criteria for each required procedure. For instance, the cavity preparation outline was graded based on external outline extension, cavity depth, direction of each wall, and cutting in sweeping curves. The assessment scores for Class II cavity preparations ranged from 0 to 75 points, while scores for Class II resin composite restorations ranged from 0 to 60 points. During the formative exercises, students assessed their performance using the provided rubrics, which allowed them to become familiar with the criteria. During the final competency examination, students were instructed to self-assess their performance immediately after completing each procedure using the same rubric. After completing their self-assessment, the students submitted their typodonts along with the completed rubric forms. All typodonts were de-identified before faculty grading.

The 6 full-time faculty members at the Department of Operative Dentistry, Faculty of Dentistry, Ain-Shams University, who taught the third-year pre-clinical operative course, were calibrated by the course director throughout the course. These faculty members (KN, MN, DM, RS, MG, and KA) participated in grading the final competency examinations. The faculty calibration level was statistically evaluated after grading the students' performance. This evaluation process was completed before any further statistical analysis took place. The inter-rater reliability (IRR) among faculty members was analyzed using the intraclass correlation coefficient (ICC) (Microsoft Excel v.16.73; Microsoft Corporation, Redmond, USA).[32]

The faculty members independently assessed each procedure using the rubric employed by the students for their self-assessments. Both student self-assessment scores and faculty grades were converted into percentages to facilitate the comparison. The mean faculty grades were considered the actual student grades. The difference between the student and faculty scores (Student-Faculty (S-F) gap) was used as a measure of the students' self-assessment skills. The S-F gap was calculated separately for cavity preparation and restoration. The relationship between the students' performance and their self-assessment skills was analyzed using Student's *t*-tests and Pearson's correlation.

In addition, the data was stratified by student performance into quartiles. Student's *t*-test was used to compare the S-F gap within each quartile to the rest of the class. The data was also divided by gender to examine the impact of gender on a student's self-assessment skills. Within each gender group, the data was further stratified into quartiles based on the students' performance to evaluate the effect of gender on the self-assessment skills of lower- and higher-performing students.

Results

A total of 335 students participated in this study. Out of the 335 students, 205 students were female (61%) and 130 students were male (39%). Six calibrated faculty graders evaluated 335 student performances of both procedures blindly and independently. The calibration level among faculty graders was analyzed, and the results indicated excellent IRR with an absolute agreement of 0.94 and consistency of 0.94 for the procedures.

The students' performance was presented as the mean value of faculty grades. The mean faculty grades for cavity preparation, restoration and for both combined were 69.0 ±12.0%, 77.2 ±11.9% and 72.6 ±10.4%, respectively. The mean student self-assessment scores for cavity preparation, restoration and for both combined were 82.9 ±8.9%, 85.4 ±9.4% and 84.0 ±7.8%, respectively. Overall, the mean S-F gaps were found to be positive, indicating that students tended to overestimate their performance compared to the faculty graders. The mean S-F gaps for cavity preparation, restoration and for both combined were 13.9 ±11.9%, 8.2 ±11.1% and 11.4 ±9.9%, respectively. Mean faculty grades, self-assessment scores and S-F gaps are presented in Table 1.

The analysis of the faculty grades, stratified by quartile, revealed statistically significant differences in S-F gaps between lower- and higher-performing students. Specifically, when comparing the S-F gaps of the lower-performing students (students in the bottom quartile) with the rest of the class, significantly higher S-F gaps were observed for preparation (25.7 ±12.5% vs. 8.11

Table 1. Mean and standard deviation for faculty grades, students' self-assessment scores and S-F gaps for all procedures ($N = 335$)

Procedure	Faculty grade	Self-assessment score	S-F gap	Top quartile S-F gap	Bottom quartile S-F gap
Class II composite preparation	69.0 ±12.0	82.9 ±8.9	13.9 ±11.9	7.4 ±7.4	25.8 ±12.6
Class II composite restoration	77.2 ±11.9	85.4 ±9.4	8.2 ±11.1	0.82 ±7.1	19.7 ±11.3
Both procedures combined	72.6 ±10.4	84.0 ±7.8	11.4 ±9.9	4.8 ±5.3	21.2 ±9.7

±7.6%, $p < 0.0001$), restoration (19.7 ±11.3% vs. 4.38 ±8.1%, $p < 0.0001$) and both procedures combined (21.2 ±9.7% vs. 8.11 ±7.7%, $p < 0.0001$). In contrast, the S-F gaps of the higher-performing students (students in the top quartile) demonstrated significantly lower gaps compared to the rest of the class for preparation (7.4 ±7.4% vs. 20.1 ±12.0%, $p < 0.0001$), restoration (0.82 ±7.1% vs. 10.5 ±11.2%, $p < 0.0001$) and both procedures combined (4.8 ±5.3% vs. 13.5 ±10.2%, $p < 0.0001$, Table 2).

The study utilized linear regression analysis to evaluate the correlation between students' self-assessment skills and their performance. The scatter plot in Fig. 1 illustrates a negative correlation between the S-F gap (students' self-assessment skills) and faculty grades (students' performance). Pearson's correlation was used to further evaluate any correlations between the S-F gaps and students' performance (Prism 8.0.1; GraphPad Software, Boston, USA). The S-F gaps were found to be negatively and moderately correlated with faculty grades in both Class II composite preparation and restoration, as well as in both procedures combined. The correlation coefficients (r) were –0.725, –0.668 and –0.707, respectively, with all p-values less than 0.0001, indicating that students with higher S-F gaps tended to receive lower faculty grades. The correlation coefficients were also analyzed within different performance quartiles. Higher-performing students (top quartile) showed weak and negative correlation coefficients (r) between the S-F gap and faculty grade; these were –0.329, –0.2492 and –0.3737 for preparation, restoration and both procedures combined, respectively. These correlation coefficients were statistically significant ($p < 0.0001$). In comparison, lower-performing students (bottom quartile) showed stronger negative correlations (r) of –0.647, –0.353 and –0.6258 (all $p < 0.0001$) for preparation, restoration and both procedures combined, respectively.

Histograms in Fig. 2A and 2B show the distribution of S-F gaps in the top and bottom quartiles of student performances. This analysis defines underestimated self-assessments as S-F gaps less than 0, accurate self-assessments as 0, and overestimated self-assessments as S-F gaps greater than 0. In the bottom quartile, while none of the students underestimated their performance and only 1.2% of students accurately self-assessed, the majority of students (98.8%) overestimated their performance. In the top quartile, 11.0% of students underestimated their performance and 9.7% of students self-assessed accurately. However, most students (79.3%) still overestimated their performance.

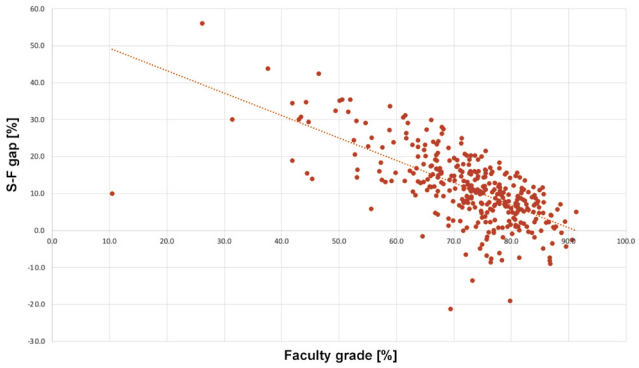

Fig. 1. Scatter plot showing the negative relationship between faculty grades and Student-Faculty (S-F) gaps ($N = 335$)

A comparative evaluation between female and male student performances and self-assessment skills also revealed some interesting findings. When compared by gender, female students demonstrated significantly higher performances compared to male students in preparation (70.7 ±10.4 vs. 66.3 ±13.8, $p = 0.0009$), restoration (80.1 ±9.1 vs. 72.7 ±14.2, $p < 0.0001$) and both procedures combined (74.9 ±8.2 vs. 69.1 ±12.4, $p < 0.0001$). On the other hand, male students showed significantly higher S-F gaps compared to female students in preparation (15.6 ±12.8 vs. 12.8 ±11.1, $p = 0.0389$), restoration (12.0 ±11.9 vs. 5.8 ±10.0, $p < 0.0001$) and both procedures combined (14.0 ±10.3 vs. 9.7 ±9.4, $p = 0.0001$) (Table 3).

Table 2. Mean and standard deviation for S-F gaps for all procedures stratified by quartile and compared to the rest of the class

Procedure	Top quartile vs. rest of the class			Bottom quartile vs. rest of the class		
	top ($n = 82$) [%]	rest ($n = 253$) [%]	p-value	bottom ($n = 83$) [%]	rest ($n = 252$) [%]	p-value
Class II composite preparation	7.4 ±7.4	20.1 ±12.0	<0.0001*	25.7 ±12.5	8.11 ±7.6	<0.0001*
Class II composite restoration	0.82 ±7.1	10.5 ±11.2	<0.0001*	19.7 ±11.3	4.38 ±8.1	<0.0001*
Both procedures combined	4.8 ±5.3	13.5 ±10.2	<0.0001*	21.2 ±9.7	8.11 ±7.7	<0.0001*

*statistically significant ($p < 0.05$).

Table 3. Mean and standard deviation for the faculty grades, students' self-assessment scores and S-F gaps for all procedures stratified by gender

Procedure	Faculty grade [%]			Self-assessment score [%]			S-F gap [%]		
	M	F	p-value	M	F	p-value	M	F	p-value
Class II composite preparation	66.3 ±13.8	70.7 ±10.4	0.0009*	81.9 ±8.2	83.6 ±9.2	0.0846	15.6 ±12.8	12.8 ±11.1	0.0389*
Class II composite restoration	72.7 ±14.2	80.1 ±9.1	<0.0001*	84.7 ±10.2	85.8 ±8.8	0.2647	12.0 ±11.9	5.8 ±10.0	<0.0001*
Both procedures combined	69.1 ±12.4	74.9 ±8.2	<0.0001*	83.1 ±7.5	84.6 ±7.9	0.0925	14.0 ±10.3	9.7 ±9.4	0.0001*

*statistically significant ($p < 0.05$). Males (M): $n = 130$; Females (F): $n = 205$.

Figures 2C and 2D present histograms that visually represent the distribution of S-F gaps for female and male students. In the analysis of male students, the majority of them (90.7%) overestimated their performance. Only 1.5% of male students underestimated their performance and 8.5% of male students accurately self-assessed their performance. In contrast, among female students, 7.8% of them underestimated their performance and 13.2% accurately self-assessed. The majority of female students (79.0%) overestimated their work.

Within each gender group, the data was further stratified into quartiles based on students' performance. Table 4 provides further insights into the impact of gender within quartiles. Female students, both in the top and bottom quartiles, had higher mean faculty grades compared to male students. These findings indicate that female students, regardless of their performance level, performed better than their male counterparts. In terms of self-assessment, both female and male students in the top quartile demonstrated similar S-F gaps, with no statistically significant difference between the genders (6.0 ±5.0 for males vs. 4.1 ±5.8 for females, p = 0.1437). However, in the bottom quartile, there was a statistically significant difference in S-F gaps between female and male students. Lower-performing male students tended to overestimate their performance more than lower-performing female students (26.1 ±10.5 for males vs. 17.6 ±9.6 for females, p = 0.0004).

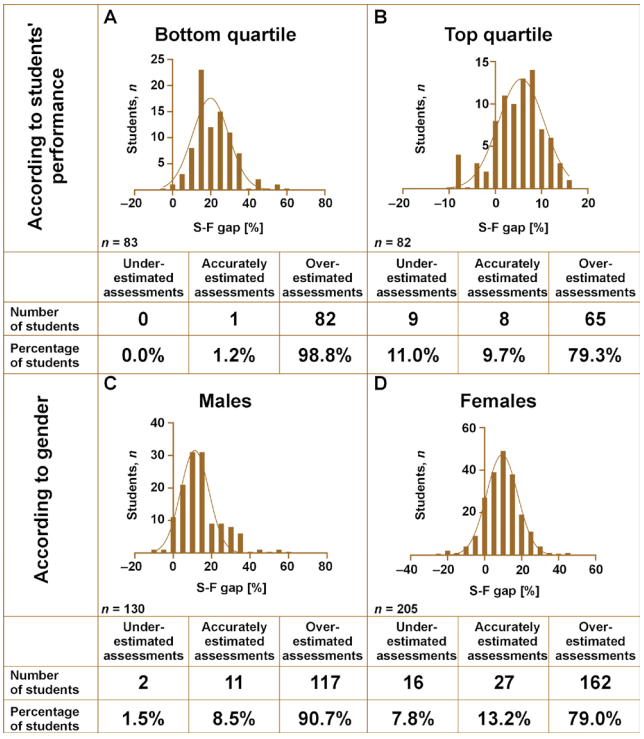

Fig. 2. Histograms showing the distribution of the S-F gap compared to the normal distribution

A. Bottom quartile students; B. Top quartile students; C. Male students; D. Female students. Underestimated self-assessments are defined as S-F gaps <0, accurate self-assessments are equal to 0 and overestimated self-assessments are defined as S-F gaps >0.

Table 4. Mean and standard deviation for faculty grades, students' self-assessment scores and S-F gaps for all procedures stratified by gender and quartile

Procedure	Top quartile faculty grade [%]			Bottom quartile faculty grade [%]			Top quartile self-assessment score [%]			Bottom quartile self-assessment score [%]			Top quartile S-F gap [%]			Bottom quartile S-F gap [%]		
	M	F	*p*-value	M	F	*p*-value	M	F	*p*-value	M	F	*p*-value	M	F	*p*-value	M	F	*p*-value
Class II composite preparation	80.9 ±3.9	82.8 ±3.1	0.0182*	47.5 ±11.0	56.9 ±7.6	<0.0001*	87.2 ±4.9	88.1 ±5.8	0.4390	77.3 ±8.7	79.5 ±10.8	0.3343	6.3 ±5.7	5.4 ±6.4	0.5288	29.9 ±13.3	22.6 ±11.7	0.0098*
Class II composite restoration	87.0 ±3.2	89.4 ±1.9	<0.0001*	52.2 ±11.7	64.8 ±7.0	<0.0001*	90.1 ±5.6	89.6 ±7.1	0.7444	76.5 ±14.4	81.7 ±11.4	0.1021	3.1 ±6.0	0.2 ±7.3	0.0678	24.3 ±3.1	16.9 ±9.1	0.0074*
Both procedures combined	81.7 ±3.6	84.4 ±2.6	0.0001*	50.8 ±9.3	64.0 ±5.2	<0.0001*	87.6 ±5.2	88.5 ±5.4	0.4423	76.9 ±9.0	81.6 ±8.8	0.0210*	6.0 ±5.0	4.1 ±5.8	0.1437	26.1 ±10.5	17.6 ±9.6	0.0004*

*statistically significant ($p < 0.05$).

Discussion

Our study showed that students tended to overestimate their performance, as evidenced by a positive mean S-F gap across all performed procedures. Linear regression and Pearson's correlation analyses revealed a negative correlation between S-F gaps and student performances, indicating that higher-performing students had lower S-F gaps, which suggests better self-assessment skills. This trend was more pronounced when the data was stratified by student performance into quartiles. Lower-performing students showed a more than 4-fold higher S-F gap than their peers, while higher-performing students demonstrated a much smaller S-F gap. Moreover, higher-performing students generally demonstrated a weaker correlation, suggesting a relatively superior ability to self-assess, whereas lower-performing students showed a stronger correlation, indicating a greater discrepancy between their self-assessment and their actual performance.

The histograms compare the distribution of the self-assessment tendencies between lower- and higher-performing students. Lower-performing students were more prone to overestimate their work, while higher-performing students showed a higher proportion of accurate self-assessments. It is noteworthy that none of the students in the bottom quartile underestimated their performance, and only 1 student accurately assessed themselves. This finding indicates that students may lack self-awareness or have difficulty recognizing areas where they need to improve their performance.

Thus, we rejected our null hypothesis that there was no difference between students' self-assessment and the grades they received from faculty. Our findings are in accordance with previous research that has also found that lower-performing students overestimate their skills compared to higher-performing individuals.[2–5] The findings from the first part of our study indicate that the pre-clinical performance of undergraduate students is influenced by their self-assessment skills. This suggests that students' self-assessment may potentially serve as an early predictor of performance during dental training.

Our study also found that students demonstrated better self-assessment abilities for restoration assignments compared to cavity preparation, as evidenced by a smaller S-F gap. This is consistent with previous studies indicating that more complex procedures, such as composite cavity preparation, may require greater knowledge and technical skills. Therefore, the students' ability to accurately evaluate their performance decrease as they are less confident of their skills.[21,28,33]

Furthermore, we examined the impact of gender on self-assessment among undergraduate students. In our study, male students were found to significantly overestimate their performance compared to their female peers, despite the fact that female students received significantly higher faculty grades. Therefore, we rejected the null hypothesis that there is no difference between male and female students in terms of self-assessment.

Interestingly, when we further analyzed the data by stratifying student performances into quartiles within each gender group, we observed that gender did not affect the self-assessment ability of higher-performing students. Students in the top quartile of both genders more accurately assessed their performances, suggesting that higher-performing students possess stronger self-assessment skills regardless of gender. However, there was a significant difference in self-assessment skills between lower-performing male and female students, with females demonstrating better assessment and performance abilities. Previous research has also found that gender differences are more pronounced among lower-performing students.[28]

The distribution patterns presented in the histograms provide support for the gender-based differences among dental students. The histograms showed that male students tend to overestimate their performance, while female students tend to either evaluate themselves accurately or underestimate their work.

Our study showed significant gender-based differences in both performance and self-assessment skills. This is consistent with previous research, which has discovered similar results concerning gender differences.[24,28] Previous studies have suggested that female students may have been taught to be more humble and modest.[29] In fact, female students have been observed to underestimate themselves despite similar performances in both low- and high-stake environments among medical and dental students, indicating the presence of systemic factors within the educational culture.[31,34] Moreover, previous studies showed that female students experience greater stress related to their confidence in becoming successful students and the difficulty in learning precision of manual skills required for pre-clinical and laboratory work.[35,36] Additionally, they tend to experience more stress related to examinations and grades.[26] To address these stressors, it is recommended that students be taught self-assessment skills to enhance their self-directed learning process and, ultimately, their performance.[20]

It is important to understand the significance of gender as an influencing factor in student performance within healthcare education. The findings from the second part of our study, which evaluated the role of gender in student self-assessment, provide valuable insights into dental education not only in Egypt, but also on a global scale.

Since students tend to overestimate their performance consistently, it is likely that this is due to their overconfidence. It has also been suggested that students may overestimate their abilities by relying on past per-

formances rather than their current work.[2] Lower-performing students may overestimate their abilities due to worse comprehension of the parameters of the assignment, leading to inaccurate self-assessments. It has been argued that lower performers likely display deficits in metacognitive skills, which results in their inflated self-assessment.[9] Kruger and Dunning suggested that both poor performance and an inability to recognize poor performance stem from overall incompetence.[9] Importantly, confidence may also contribute to this issue, as these students may struggle more with being perceived as lower-achieving and therefore overcompensate with their self-assessment scores. Our study examined the initial exposure to pre-clinical exercises for third-year students in the operative dentistry course. Previous studies have found that self-assessment is a skill that develops over time and can be influenced by the stage of a student's education during which it is evaluated.[8,30]

Our study highlights the importance of improving student self-assessment skills. This can be achieved by emphasizing the significance of self-assessment to students and fostering a shared understanding of necessary competencies between students and faculty. Students should be encouraged to reflect on their performance before receiving faculty feedback to emphasize the significance of developing critical judgment skills in their own work. Peer learning has also been found to be very effective at strengthening self-assessment skills and should be implemented.[24,33] Moreover, our findings support the importance of a more balanced education from an early age to reduce gender differences in confidence and self-awareness. Further exploration of factors that influence students' self-assessment, including gender, will play an important role in creating a more equitable and inclusive educational environment.

Providing appropriate training to faculty members is essential to ensure consistent and reliable teaching and grading of student performance. The study demonstrated an excellent IRR of our faculty grading, with an absolute agreement of 0.94 and consistency of 0.94 for all procedures, indicating that the faculty graders were well-calibrated and in agreement. This enhances the accuracy and consistency of the grading process. Evaluating the calibration levels of participating faculty members should be considered a valuable measure to maintain the quality of assessment and education.

Although our study had a large sample size, it was conducted on a single class at a single institution, which limits the generalizability of our findings. Future studies should examine multiple classes across various disciplines and educational centers to draw broader conclusions. It would also be beneficial to assess students' progress throughout the academic year. Despite its limitations, this study is a valuable contribution to global dental education research as it evaluates students' self-assessment skills and identifies influencing factors.

Conclusions

Accurately assessing clinical performance is crucial for dental students as they will mostly work as solo practitioners in their future clinical practice. The study findings indicate that most students tend to overestimate their performance, with higher-performing students demonstrating significantly more accurate self-assessments than lower-performing students. Furthermore, male students had lower performance yet significantly overestimated their work compared to their female peers. This study provides valuable insight into the self-assessment skills of undergraduate students and should lead to further investigation into effective interventions and educational strategies aimed at enhancing students' self-awareness and their ability to accurately assess their performance.

Ethics approval and consent to participate

The study was approved by the ethics committee of the Faculty of Dentistry of Ain Shams University, Cairo, Egypt (IRB approval No. FDASU-Rec IR092206).

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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Original paper

Systemic administration of cerium oxide nanoparticles reduces oxidative stress in young patients with generalized gingivitis and obesity

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Abstract

Background. Obesity and periodontal diseases are associated with oxidative stress activation. Periodontitis and gingivitis are inflammatory diseases that cause systemic and local production of reactive oxygen species, leading to tissue damage. Obesity exacerbates systemic free radical oxidation.

Objectives. The aim of the study was to evaluate the influence of cerium oxide nanoparticles (CNPs) as potential pharmaceutical agents for targeting oxidative stress activation in patients with obesity and periodontal disease.

Material and methods. Young patients with obesity and generalized gingivitis were randomly allocated into 2 groups. In the first group ($n = 28$), a professional oral hygiene procedure was performed, followed by the local application of the Nanosept solution (a formulation comprising CNPs and chlorhexidine digluconate (CHG)). Over the next 5 days, the subjects were instructed to administer the Nanosept solution twice a day on the gums using a cotton sponge for 5 min. The second group ($n = 30$) was additionally prescribed the antioxidant Cerera (CNPs) for 10 days.

Results. Following treatment in both groups, a complete resolution of gingivitis was registered. In both groups, a significant decrease in salivary mucopolysaccharides and total nitric oxide synthase (NOS) activity was observed. However, a significant decrease in oxidative and nitrosative stress markers, as well as an increase in catalase activity were registered only after systemic administration of CNPs. Similarly, the normalization of colonization resistance (CR) was observed only in the second group.

Conclusions. Systemic administration of CNPs in the treatment of obese patients with generalized gingivitis resulted in a decrease in oxidative and nitrosative stress activation in the oral cavity, enhanced antioxidant capacity of the saliva, and normalized the level of CR of the oral cavity.

Keywords: obesity, periodontitis, cerium oxide nanoparticles, nanozyme, clinical trial

Highlights

- Both treatment modalities (local cerium oxide nanoparticles (CNPs) + chlorhexidine digluconate (CHG) and combined local/systemic CNPs) achieved complete resolution of generalized gingivitis within 1 week.
- Combined local/systemic CNPs restored colonization resistance more effectively than local treatment alone.
- Salivary inflammation markers (free fucose, glycosaminoglycans) decreased significantly with both treatments.
- Systemic CNPs reduced oxidative stress and enhanced antioxidant activity.
- No adverse effects were observed, and systemic CNPs provided additional benefits for patients predisposed to oxidative and nitrosative stress, including those with obesity.

Introduction

The prevalence of periodontal diseases is extremely high. The incidence of gingivitis is estimated to range from 50% to 100% among young individuals from different populations.[1–3] Over the past 30 years, the prevalence of periodontitis has notably increased, especially among younger individuals.[4] Unmanaged gingivitis has a tendency to progress into periodontitis, particularly in individuals with chronic conditions that affect host immune responses such as diabetes mellitus, obesity, autoimmune conditions, and endocrine diseases. Also, it can increase the risk of cardiovascular disease, among others.[5–9]

Obesity has been identified as a significant predisposing factor for the development of periodontitis and an element contributing to its severity.[10–12] Young patients who are overweight or obese exhibit a higher prevalence of periodontal diseases compared to individuals with a healthy body mass index (BMI).[13,14]

One of the main pathophysiological mechanisms underlying periodontal tissue alterations is the activation of oxidative stress on a local and systemic level.[15] In obese individuals, systemic oxidative stress is caused by the secretion of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6 and leptin into the blood by excess visceral and subcutaneous adipocytes.[16] Systemic factors, including hyperglycemia, elevated tissue lipid levels, vitamin and mineral deficiencies, hyperleptinemia, increased muscle activity to support excessive weight, endothelial dysfunction, impaired mitochondrial function, and diet contribute to the development of systemic oxidative stress.[8,17–21] Local activation of nitrosative and oxidative stress in the periodontium is caused by the response of polymorphonuclear leukocytes to gram-negative bacterial lipopolysaccharide, leading to respiratory burst and the secretion of inflammatory mediators. Excessive production of inducible nitric oxide synthase (iNOS) by neutrophils results in increased levels of nitric oxide (NO) and peroxynitrite ($ONOO^-$).[22–24]

Cerium oxide nanoparticles (CNPs) exhibit strong enzyme-like activity similar to that of catalase and superoxide dismutase enzymes.[25,26] Compared with traditional non-enzyme antioxidants, such as vitamin C, vitamin E, ethylenediaminetetraacetic acid (EDTA), and quercetin, which can take part only in 1 redox cycle, CNPs show a capacity for self-regeneration. Within a few days, these particles are ready to neutralize an additional superoxide radical.[27]

Due to their antioxidant properties, CNPs are widely used in the treatment of diseases and conditions associated with the overproduction of oxidative radicals, such as wound healing,[28] oral cavity inflammatory diseases,[29,30] cancer,[31] periodontitis,[32] and acute kidney injury.[33] Recent studies have shown that, in combination with chlorhexidine digluconate (CHG) solution, CNPs significantly improve the antimicrobial properties of the composition.[34] Nanoparticle-based treatment has become a prevalent practice in dentistry. Soundarajan and Rajasekar demonstrated that graphene oxide–silver nanocomposite mouthwash exhibited bactericidal and anti-inflammatory properties in the treatment of gingivitis.[35]

Our study aimed to evaluate the influence of local and systemic administration of CNPs on targeting oxidative stress activation in the oral cavity and on enhancing antioxidant properties of saliva in young individuals with obesity and generalized gingivitis.

Material and methods

Trial design

The randomized controlled, parallel clinical trial was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines.[36] Participants were allocated to groups using a randomized assignment process. The study design is presented in Fig. 1.

Participants

The recruitment of patients was performed during the regular check-up of students. Before the study, all participants were informed about its design, purpose and possible risks. The eligibility criteria were as follows: young age (18–22 years); BMI of 30 kg/m^2 and higher; Caucasian ethnicity; non-smoking status; absence of diagnosed

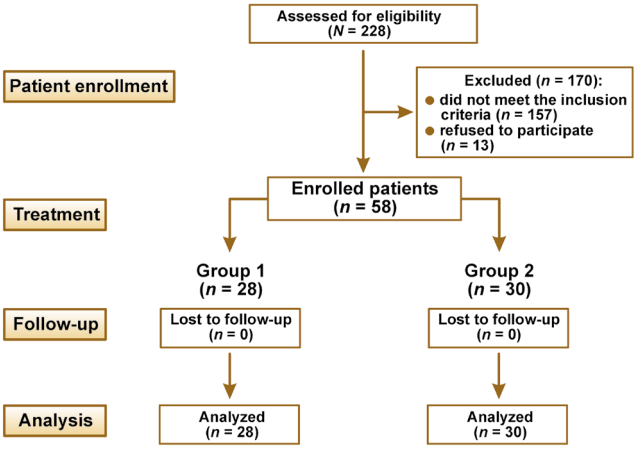

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study

endocrine and cardiovascular diseases; presence of at least 6 teeth per quadrant (excluding wisdom teeth); and a diagnosis of dental plaque-induced generalized gingivitis (mediated by the systemic risk factor of obesity), based on the 2017 Classification of Periodontal and Peri-implant Diseases and Conditions.[10] The exclusion criteria were pregnancy, allergy to biguanides, the presence of removable or fixed prosthetic appliances or brackets, local dental plaque biofilm retention factors such as prominent restoration margins, and the use of antibiotics or antimicrobial mouthwashes during the preceding 3 months.

All participants were patients of the University Clinic of the Department of Therapeutic Dentistry at Poltava State Medical University in Ukraine. The data was collected from March 2021 to February 2022.

Interventions

The BMI measurements were used to determine the distribution of individuals into the treatment groups. Additionally, a full-mouth periodontal chart was completed for each participant. Two dentists (MSkr and TP) performed clinical measurement of periodontal parameters using the automated computer detecting system pa-on Parometer® (orangedental, Biberach, Germany). Furthermore, the Greene–Vermillion oral hygiene index (OHI), the approximal plaque index (API), the papillary-marginal-alveolar index (PMA), the papillary bleeding index (PBI), and clinical attachment loss (CAL) were considered.

The patients were randomly allocated into 2 groups. The first group (n = 28) received the treatment for dental plaque-induced gingivitis mediated by obesity, which entailed the following: complete removal of dental plaque and tartar via ultrasound scaling (Mini Piezon SA CH-1260; EMS Electro Medical Systems S.A., Nyon, Switzerland); polishing of the oral and vestibular teeth surfaces with an Enhance® finishing cup (Dentsply Sirona, Bensheim, Germany); and interproximal surface polishing with a narrow, fine grit polishing polyester strip (GC Corporation, Tokyo, Japan). The Nanosept solution, an antiseptic formulated for the local treatment of periodontal diseases (patent No. 139875, valid from January 27, 2020), was administered as a local antimicrobial treatment. The Nanosept solution is a 0.05% solution of CHG (Chervona Zirka, Kharkiv, Ukraine) and CNPs (2–7 nm), stabilized with sodium citrate, with a final concentration of 70 μg/mL. Subsequently, the oral hygiene Nanosept solution was applied to the gums with cotton sponges for 5 min. After treatment, the patients were instructed to apply the Nanosept solution twice a day (morning and evening) for 5 days after flossing and brushing their teeth, adhering to the same technique.

The second group (n = 30) underwent the same treatment as the first group, with the addition of the antioxidant Cerera (CNPs (2–7 nm), stabilized with sodium citrate). Cerera was prescribed as a general treatment for the patients, who were instructed to use it in the morning once a day by dissolving 20 drops of the antioxidant in 50 mL of drinking water for 10 days. For the purposes of this study, Cerera was synthesized and provided by MSp. The antioxidant was registered in Ukraine as a biological active supplement (registration No. TYY 10.8-2960512097-004:2015).

The follow-up period for periodontal treatment was established as 1 month. However, the participants were instructed to inform general practitioners (IS and RS) about adverse effects observed during the 9-month observation period after treatment. No adverse effects related to the provided treatment were reported.

Outcomes

The clinical data was recorded at the following time points: before treatment (T0); 7 days after treatment (T1); and 1 month after treatment (T2). For each patient, samples of the whole unstimulated saliva were collected at 2 time points: T0 and T1. The samples were procured during the morning hours, from 8.00 A.M. to 10.00 A.M. The whole saliva was collected into the test tube via passive drooling for 7 min. Then, the saliva was centrifuged for 5 min at 3,000 rpm. The supernatant was retrieved, divided into 1.0-mL aliquots and stored at –30°C until use. Oral swab samples for colonization resistance (CR) were obtained at T0 and T1.

Sample size

The sample size was calculated according to the recommendations for cross-sectional studies using the Sample Size Calculator (https://www.gigacalculator.com/calculators/power-sample-size-calculator.php). The minimum size of each group was calculated to be 29, with a 95% confidence interval (CI), type I error rate (α) of 5%, and a margin of error of 85%.

Patients were randomly allocated according to the random number table, employing the blocking type method with a block size of 4 and a range of numbers from 1 to 100. The table was designed by one of the researchers (MSki), who was not initially aware of the clinical trial design. After randomization, the sample comprised 12 male and 16 female subjects in the 1st group, and 16 male and 14 female participants in the 2nd group.

One researcher (KN) generated the random allocation sequence, 4 researchers (TP, MSkr, IS, and RS) enrolled participants, and 2 (TP and MSkr) assigned participants to interventions.

Analysis of salivary biomarkers

The determination of the free fucose content in the saliva of patients was performed according to the method designed by Sharaev et al.,[37] based on the photometry of the chromogen that is formed under the sequential exposure of fucose to sulfuric acid and cysteine sulfate. The optical density of the samples was evaluated on a spectrophotometer at a wavelength of 396 nm and 430 nm against blank samples. Then, the difference in extinction (E) between the experimental and standard samples (E = E396 – E430) was calculated and analyzed.

The content of glycosaminoglycans (GAGs) in the saliva was determined according to the method described by Sharaev et al.,[38] which is based on the property of hexuronic acids to transform into furfural aldehyde or its homologues when heated with strong mineral acids, which results in their polymerization with carbazole. Photometry was performed at a wavelength of 530 nm against concentrated sulfuric acid containing 0.2 M of sodium tetraborate.

The determination of oxidatively modified proteins (OMPs) in the saliva of patients was performed according to the spectrophotometric method based on the quantitative analysis of carbonyl groups, which are formed during the interaction of reactive oxygen species with amino acid residues. The analysis uses 2,4-dinitrophenylhydrazine.[39]

The total activity of NOS was determined by observing the difference in the concentration of nitrite ions (NO_2^-) before and after the incubation with saliva in a medium containing L-arginine (substrate of NOS) and reduced nicotinamide adenine dinucleotide phosphate (NADP). The concentration of NO_2^- was determined by the formation of diazo compounds in the reaction with sulfanilic acid. Subsequently, the reaction with α-naphthylethylenediamine was carried out, which resulted in the formation of red derivatives (azo dyes).[40] The intensity of the solution's color is proportional to the concentration of nitrites.

Thiobarbituric acid reactive substances (TBARS) in saliva were evaluated according to the method outlined by Stalnaja and Garishvili.[41] Upon heating with aldehydes, 2-thiobarbituric acid forms a trimethine complex, which exhibits a light absorption maximum at 532 nm. In this case, the intensity of the color of the solution was proportional to the concentration of TBARS.

The catalase activity in saliva was assessed according to the method described by Koroliuk et al.[42] The reaction was initiated by adding saliva to 0.003% hydrogen peroxide solution. The reaction was stopped by adding 1 mL of 4% ammonium molybdate solution. The color intensity of the solution was determined at a wavelength of 410 nm.

The proteolytic activity of saliva was calculated based on the increase in free amino nitrogen, which is formed during the hydrolytic cleavage of protein substrates. Amino nitrogen yields a blue color in the reaction with ninhydrin. The color intensity is directly proportional to the content of free amino acids against the standard, which is glycine. The determination of proteinase inhibitors is based on the measurement of the difference between the activity of the test sample, which contains a certain amount of trypsin, and the activity of the sample with saliva.[43]

The activity of α-amylase in the saliva was measured using the α-amylase kit (Filisit-Diagnostika LLC, Dnipro, Ukraine) according to the Caraway method. In the presence of α-amylase, starch is hydrolyzed to derivatives that do not give a color in reaction with iodine. The change in the color intensity of the iodine–starch complex is proportional to the activity of the enzyme in the test sample.

The activity of nitrate and nitrite reductases in saliva was determined according to the method designed by Akimov and Kostenko.[44] Enzyme activity was assessed based on the difference in the concentration of nitrites and nitrates before and after incubation of saliva in the aqueous solution of nicotinamide adenine dinucleotide (NADH). The concentration of nitrites was determined by the observation of the diazo compounds formed in the reaction with sulfanilic acid. The reaction was then carried out with α-naphthylamine (Griess–Ilosvay reagent). The color intensity of the red derivatives (azo dyes) is proportional to the nitrite concentration.

Colonization resistance of the oral mucosa

The degree of CR of the oral mucosa was determined by conducting a microscopical examination of the buccal epithelium. This method involves the determination of the adhesive number (AN) of streptococci adhered to 1 epitheliocyte, the adhesive index (AI) which quantifies the percentage of epitheliocytes that have more than 10 streptococci adhered, and a qualitative assessment of CR, denoted as the colonization resistance index (CRI). The AN < 20 and AI < 50% indicated the suppression of CR and reduced antagonistic properties of oral microflora. The CRI of 1, AN in the range of 20–60, and AI > 50% signified a high level of CR of the oral cavity, whereas the CRI of 2, AN > 60 and AI of 100% revealed an increased tension of the colonization barrier.[45] The microscopic examination of the buccal epithelial samples was performed using a light microscope (Olympus CX23 RFS1; Olympus Corporation, Tokyo, Japan) at ×400 magnification.

Statistical analysis

The GraphPad Prism v. 8.0.1 software (GraphPad Software, Boston, USA) was used for the statistical analysis of the data. The results were described as mean (*M*) and standard deviation (*SD*). One-way analysis of variance (ANOVA) was utilized to process the data from unrelated samples, with Bonferroni correction applied for multiple comparisons. Paired comparisons within a group, before and after treatment, were conducted using Student's paired *t*-test. To analyze statistical differences between different groups, an unpaired *t*-test with Welch's correction was used. The differences between the groups were considered statistically significant at $p < 0.05$.

Results

Clinical evaluation of oral hygiene level and the intensity of inflammation in the periodontium

In both groups, the initial level of oral hygiene (T0), as determined by OHI, was assessed as average. However, the API-based evaluation of the hygiene of the approximal surfaces revealed suboptimal conditions in both groups. The oral bleeding indexes that reflect the severity of inflammation (PMA and PBI) were high, indicating the presence of gingivitis, while no CAL was reported. One week after treatment (T1), the normalization of oral hygiene and the resolution of gingivitis were observed in both groups. A significant decrease in OHI, PMA and PBI was documented. After 1 month (T2), no signs of gingivitis were registered; however, plaque accumulation was high on the vestibular (OHI) and interproximal surfaces (API) (Table 1).

Changes in the colonization resistance of the oral cavity

Before treatment (T0), CRI values of 1 and 0 prevailed in both groups (Fig. 2). After treatment (T1), the majority of patients in group 1 exhibited CRI of 0 (71.43%), while the remaining individuals demonstrated CRI of 1 (Fig. 2A). However, in group 2, the value of 1 was predominant (70.00%) after treatment, with CRI of 0 being observed in 23.33% of the participants (Fig. 2B). Before treatment, AI and AN in the first group were 18.25 ±14.81 and 67.00 ±31.65, respectively, and in the second group, 19.54 ±12.53 and 64.67 ±32.81, respectively. After the treatment, a statistically significant change in AN was observed in both groups (38.96 ±37.98 and 39.23 ±33.63 in group 1 and 2, respectively; $p < 0.01$). However, changes in AI were not significant (17.46 ±14.09 and 23.21 ±19.33, respectively). After treatment (T1), no differences in AI and AN were noted between the 2 groups.

Changes in salivary biomarkers

In both groups, treatment resulted in a significant decrease in total NOS activity and free fucose concentration (Fig. 3A,F). There was no change in the levels of salivary α-amylase activity, total proteolytic activity and proteinase inhibitors, as well as the concentration of nitrites

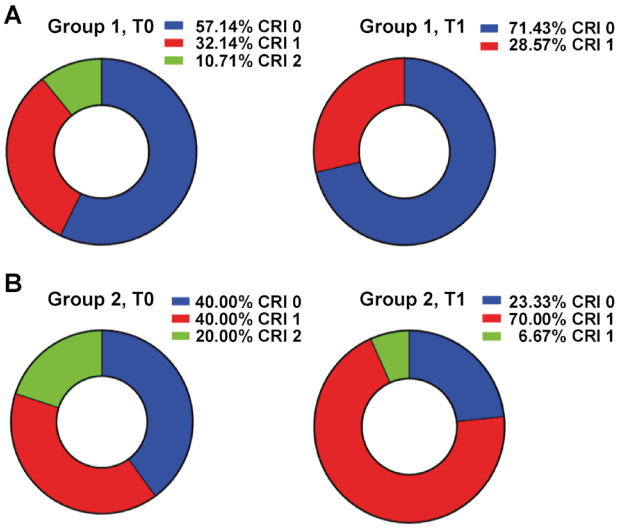

Fig. 2. Colonization resistance (CR) of the oral cavity

A. Prevalence of patients with the colonization resistance index (CRI) values of 0, 1 and 2 before (T0) and after (T1) local treatment with the Nanosept solution; B. Prevalence of patients with CRI values of 0, 1 and 2 before (T0) and after (T1) local treatment with the Nanosept solution and systemic administration of Cerera.

Table 1. Clinical assessment of oral hygiene and inflammatory indexes

Variable	Group 1			Group 2		
	T0	T1	T2	T0	T1	T2
OHI	0.83 ±0.44	0.17 ±0.24****	0.87 ±0.55$^{\#\#\#\#}$	0.92 ±0.49	0.23 ±0.23****	0.87 ±0.50$^{\#\#\#\#}$
API [%]	4.30 ±7.15	20.53 ±12.60	12.11 ±22.11$^{\#\#\#}$	6.55 ±8.82	20.50 ±12.60	10.87 ±16.60
PMA	18.52 ±6.40	2.66 ±3.65****	3.20 ±4.30	21.09 ±7.55	3.10 ±3.80****	3.90 ±4.10
PBI [%]	22.10 ±16.10	2.80 ±6.00***	8.20 ±11.10	19.00 ±4.50	1.80 ±4.50***	8.70 ±10.70$^{\#}$

OHI – Green–Vermillion oral hygiene index; API – approximal plaque index; PMA – papillary-marginal-alveolar index; PBI – papillary bleeding index; T0 – before treatment; T1 – 7 days after treatment; T2 – 1 month after treatment.

Data presented as mean ± standard deviation (*M* ±*SD*). Statistical significance between T0 and T1: *** $p < 0.001$; **** $p < 0.0001$ (one-way ANOVA). Statistical significance between T1 and T2: $^{\#}p < 0.05$; $^{\#\#\#}p < 0.001$; $^{\#\#\#\#}p < 0.0001$ (one-way ANOVA).

and nitrates in saliva (supplementary materials – available on request from the corresponding author). In the saliva of the patients from group 2, a slight yet significant decrease in nitrate and nitrite reductase was observed (Fig. 3B). A significant reduction in markers of oxidative stress, such as OMPs and TBARS, and an increase in catalase activity was detected in group 2 (Fig. 3C). A significant decline in GAG concentration was documented in group 2; however, a tendency toward a decrease in GAG concentration was also observed in group 1 (Fig. 3D).

During the observation period, no side effects of the proposed treatment were reported.

Discussion

Clinical findings

Our findings demonstrate that local treatment with the Nanosept solution, which is a combination of CNPs and CHG, and complex treatment that included a systemic application of CNPs resulted in a complete resolution of gingivitis in both groups. The results of treatment were stable up to 1 month of observation (Table 1). Oral hygiene indexes (OHI, API) returned to their initial state 1 month after treatment; however, no gingivitis was

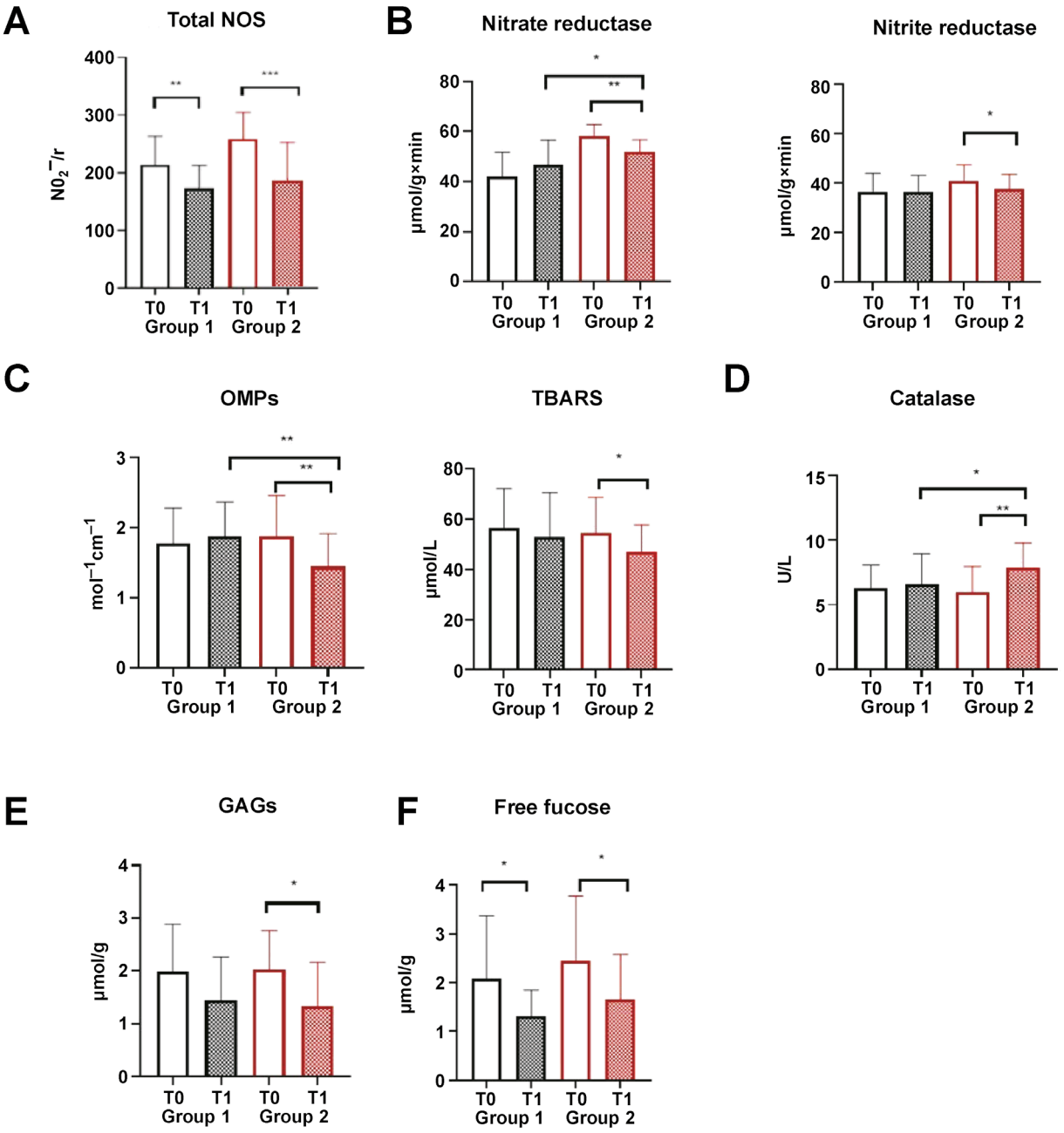

Fig. 3. Activity of salivary biomarkers and enzymes before and 7 days after treatment

A. Total salivary nitric oxide synthase (NOS) activity; B. Salivary nitrate and nitrite reductase activity; C. Levels of salivary oxidatively modified proteins (OMPs) and thiobarbituric acid reactive substances (TBARS); D. Salivary catalase activity; E. Salivary glycosaminoglycan (GAG) concentration; F. Salivary free fucose concentration. Statistical significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Student's *t*-test). Data presented as mean ± standard deviation (*M* ±*SD*).

observed. The outcomes of recent observational studies have shown that frequent toothbrushing was negatively associated with BMI.[46,47]

Colonization resistance of the oral cavity

Colonization resistance (bacterial interference) is the local mechanism of the oral cavity's unspecific immunity. It provides individual specificity and stability for the adhesion and growth of pathogen bacteria on host oral mucosae. A certain role in this process can be attributed to resident bacteria, which act as antagonists to pathogenic and opportunistic bacteria.[48] The antagonistic effect of microflora physiology is facilitated by the significant adhesive and colonizing capabilities of resident bacteria, as well as the production of specific substances, including bacteriocins and antibiotics, which suppress the growth of pathogenic microorganisms.[49,50] Several factors have an influence on CR, such as hyposalivation, changes in the qualitative composition of saliva, smoking, tobacco chewing, high sugar consumption, immunosuppression, or acidic pH.[51]

Decreased CR can lead to bacterial invasion of underlying tissues and the subsequent development of purulent-inflammatory processes.[52] Thus, germ-free animals, which lack an indigenous microbiome after transient bacterial exposure, developed intestinal walls, villi malfunction, poor nutrient absorption, vitamin deficiencies, and cecum enlargement.[53] Streptococcus species are the most prevalent microorganisms in the oral cavity and play an important role in establishing and shaping the oral microbiome.[54]

In group 1, where CNP and CHG solution was applied locally, a decrease in CR tension was observed. Patients with CRI of 2 were not registered after treatment, and CRI of 0 was predominant in the group. This finding indicates the suppression of CR and reduced antagonistic properties of oral microflora (Fig. 2A). The adhesive number decreased significantly in group 1, indicating a reduction in CR and the total microbial load of the oral mucosa. In group 2, where CNPs were applied locally and administered intraorally after treatment, CRI values of 1 prevailed among subjects, indicating a high level of CR of the oral cavity (Fig. 2B). However, approx. 30% of patients exhibited CRI of 0 after treatment, which was a sign of suppressed CR, and CRI of 2, which indicated an increased tension of the colonization barrier. Thus, systemic administration of CNPs led to an increase in CR of the oral mucosa in obese subjects with gingivitis, in comparison to group 1 where only local administration of CNPs and CHG was implemented. A significant reduction in total AN was observed following treatment in both groups, which can be attributable to high antimicrobial activity of CNP and CHG composition. However, the precise mechanism underlying the increase in CR after systemic administration of CNPs remains unclear.

Reduction in oxidative and nitrosative stress after systemic administration of CNPs

Periodontal tissue damage, determined through oxidative and nitrosative stress, has been identified as an important mechanism in the development of periodontitis, especially in patients with obesity.[15,18,19,21] Nitrate (NO_3^-) and nitrite (NO_2^-) reductase, vital enzymes within the nitrate–nitrite–NO pathway, facilitate NOS and subsequent vasodilation. These enzymes act as substrates for the synthesis of NO under physiological conditions or in response to hypoxia, inflammation and diseases involving ischemia–reperfusion injury.[55] Oral cavity bacteria, predominantly those located at the back of the tongue, also play a significant role in the conversion of NO_3^- and NO_2^- to NO in the human body. Up to 25% of nitrate in circulation is excreted by salivary glands, resulting in a 20-fold increase in its concentration in saliva.[56] The activity of nitrate and nitrite reductase and NO as their end product significantly increases in patients with periodontitis.[57] High activity of iNOS in saliva and periodontal tissues is a marker of periodontitis, and it is much higher in patients with aggressive periodontitis.[23,58] Excessive activation of iNOS (mostly by mast cells and lymphocytes)[23] as well as nitrate and nitrite reductases in obese individuals can be described as a compensatory reaction to hypoxia that leads to NOS and vessel dilatation.[15] Hyperproduction of NO causes nuclear factor kappa B (NF-κB) activation that triggers the synthesis of systemic inflammatory response cytokines. A reaction of NO with superoxide anion radical results in $ONOO^-$ formation and free radical cell damage, necrobiosis, and premature aging. Xu et al. and Hou et al. reported that CNPs decrease the nitrate reductase activity in bacterial biofilms.[59,60] Cerium nanoparticles convert both superoxide (O_2^-) and H_2O_2 into more inert species, while also scavenging NO both in vitro and in vivo, significantly accelerating the decay of $ONOO^-$.[61]

A significant decrease in total salivary NOS levels was observed after treatment in both groups ($p < 0.01$ in group 1 and $p < 0.001$ in group 2) (Fig. 3A). The activity of nitrite and nitrate reductases decreased only after peroral administration of CNPs (group 2). The nitrate reductase activity following treatment was significantly higher in group 2 compared to group 1 ($p < 0.05$), which might stem from the higher initial activity of this enzyme in group 2 (Fig. 3B). The concentration of the substrate (nitrites and nitrates) remained constant before and after treatment in both groups.

The total concentration of biomarkers of oxidative stress, TBARS and OMPs, decreased significantly only after systemic administration of CNPs (group 2) (Fig. 3C). In group 2, a significant increase in catalase activity was observed ($p < 0.01$) (Fig. 3D). The concentration of OMPs in group 2 was significantly lower than that in group 1 ($p < 0.01$) (Fig. 3C). Additionally, a notable increase in catalase activity

was observed in group 2 following treatment ($p < 0.05$) (Fig. 3D). The inhibition of oxidative stress and an increase in the antioxidative capacity of saliva after the intraoral administration of CNPs can be attributed to the CNP catalase activity and superoxide dismutase (SOD) mimetic activity.[62,63] However, local application of CNPs (group 1) did not result in the suppression of oxidative stress.

Glycosaminoglycans (dermatan sulfate, heparan sulfate, keratan sulfate, and creatine) are components of the connective tissue extracellular matrix. A high concentration of GAGs in saliva has been associated with inflammatory periodontal diseases, oxidative and nitrative stress activation, mucositis, wound healing, and ageing.[64–67] Periodontopathogens, such as *Tannerella forsythia*, secrete sialidases that enzymatically digest the mucopolysaccharides present in the gingival extracellular matrix. This process leads to the release of GAGs, which promotes further bacterial colonization and the development of the climax biofilm.[68,69] In the current study, the concentration of GAGs in saliva decreased after treatment in both groups (Fig. 3E), which can be attributed to the complete resolution of gingivitis and oral hygiene improvement.

An increase in the free fucose concentration in saliva is associated with oral mucosa and gingiva inflammation. Free fucose is released through the hydrolytic activity of pathogens and indigenous bacteria.[70,71] High salivary free fucose levels create favorable conditions for the colonization of opportunistic bacteria, which can utilize it as an energy source.[71]

In both groups, treatment resulted in a significant decrease in the salivary fucose and GAG concentrations, consequently leading to a reduction in CR tension (Fig. 3E,F).

Patients suffering from obesity and periodontal diseases should receive local traditional non-surgical treatment for gingivitis as well as pharmaceutical treatment, which will target the pathogenesis of both periodontal disease and obesity. This would mediate host immune response and result in a more severe course of gingivitis. It is important to stop the progression of gingivitis and prevent irreversible changes such as alveolar bone loss or CAL, which effectively contribute to the paradigm shift from reactive medicine to the advanced approach by utilizing predictive, preventive and personalized medicine (3PM) concepts.[72]

Limitations

A limitation of the study is the inability to control the regularity, proper duration and performance of all prescribed treatment procedures performed by patients at home. Another constraint is a relatively short post-treatment periodontal observation period (1 month). However, a 9-month observation period was established to monitor for any side effects that may have ensued from the treatment.

A group of patients receiving standard gingivitis treatment and a second group receiving local chlorhexidine application in addition to the standard treatment may be suitable subjects for this study. However, establishing two additional groups would have required recruiting more patients. This proved unfeasible, as all available participants were medical students from our university. In order to ensure the unification of environmental factors and lifestyles, we decided not to recruit participants who were not medical students at Poltava State Medical University. The severity (percentage of affected sites) and location of inflammatory sites of generalized gingivitis exhibited variability among all patients. The study participants comprised medical and dental students. It is noteworthy that the participants' enrollment in the clinical study occurred in close temporal proximity to their examination session. This period is associated with elevated academic stress, which has been demonstrated to cause the activation of the hypothalamic–pituitary–adrenal axis, oxidative stress and gingivitis.

Conclusions

Both local (CNPs and CHG) and complex treatment resulted in a complete resolution of generalized gingivitis. In both groups, a significant reduction in AN of oral streptococci on the buccal epithelium was observed. After the local treatment with CNPs, a decline in CR was observed in group 1. However, the combination of local and systemic CNP administration resulted in a restoration of the normal level of CR. Both treatment modalities led to a decrease in salivary free fucose and GAG concentration, which are the markers of oral mucosa and gingiva inflammation, and as a substrate can contribute to extensive colonization of the oral cavity with opportunistic bacteria. The total NOS activity significantly decreased in both groups. Yet, a significant reduction in nitrite and nitrate reductases was observed only after the intraoral administration of CNPs. This was accompanied by a significant reduction in salivary oxidative stress biomarkers (OMPs and TBARS) and a significant increase in salivary catalase activity. In comparison with the local treatment alone, systemic administration of CNPs resulted in a significant increase in salivary catalase activity and a decrease in OMP level in saliva after treatment.

Therefore, as an antioxidant, CNPs can be used for the complex treatment and prevention of periodontal diseases, especially in patients with predisposed conditions associated with oxidate and nitrosative stress activation, such as obesity.

Ethics approval and consent to participate

The clinical trial design was approved by the Committee on Ethical Issues and Biomedical Ethics of Poltava State Medical University, Ukraine (approval No. 197).

All procedures performed in this study were in accordance with the ethical standards of the institutional and Ukrainian national research committees (statement of the Ministry of Health of Ukraine No. 690 revised in 2009) and with the Helsinki Declaration (as revised in 2013). Written informed consent was obtained from all patients.

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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Original paper

Analysis of potential risk factors for the development of medication-related osteonecrosis of the jaw

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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. Medication-related osteonecrosis of the jaw (MRONJ) is an undesirable consequence of the action of drugs, involving the exposure of bones in a patient not exposed to ionizing radiation in the head and neck area. The occurrence of MRONJ is associated with therapy with anti-resorptive drugs (bisphosphonates), receptor activator of nuclear factor-kappa B ligand (RANKL) drugs, e.g., denosumab, and anti-angiogenic drugs that are vascular endothelial growth factor (VEGF) inhibitors.

Objectives. The aim of the present study was to identify risk factors for the development of MRONJ.

Material and methods. The medical records of patients hospitalized in the years 2015–2022 in the Department of Maxillofacial Surgery of the Ludwik Rydygier Specialist Hospital in Krakow and the Clinical Department of Maxillofacial Surgery of University Hospital in Krakow, Poland, were retrospectively analyzed. The study included patients treated for MRONJ in the maxilla and/or the mandible with a history of past bisphosphonate therapy. Patients with symptoms of osteonecrosis after radiotherapy of the head and neck region were excluded from the study. The patients' demographic data, comorbidities, the initial disease treated with bisphosphonates, the route of drug administration, the type of causative dental surgery, the area of necrosis, and the MRONJ class according to the American Association of Oral and Maxillofacial Surgeons (AAOMS) criteria were analyzed.

Results. The investigated group consisted of 29 females and 14 males. Common comorbidities were anemia, diabetes mellitus (DM) and hypertension. Bisphosphonates were used in 30 patients (69.8%) treated for cancer, in 10 patients (23.3%) treated for osteoporosis and in 2 patients (4.7%) treated for osteopenia. In the majority of cases ($n = 19$; 44.2%), bisphosphonates were administrated intravenously. Medication-related osteonecrosis of the jaw was diagnosed in the mandible in 25 cases (58.1%) and in 18 (41.9%) – in the maxilla. In 14 patients (32.6%), necrosis was initiated by a dental procedure, most often tooth extraction.

Conclusions. Risk factors for the development of MRONJ in patients treated with bisphosphonates include the intravenous route of drug administration, past intraoral surgery, female gender, and senior age.

Keywords: therapy, diagnosis, risk factors, MRONJ, bisphosphonate-associated osteonecrosis of the jaws

Highlights

- Preventing medication-related osteonecrosis of the jaw (MRONJ) involves educating both patients and dentists about the risks and proper management strategies, especially when performing intraoral procedures.
- Before initiating bisphosphonate therapy, it is essential to conduct a thorough oral cavity examination to identify and eliminate any potential sources of inflammation or infection.

Introduction

Medication-related osteonecrosis of the jaw (MRONJ) is an undesirable consequence of the action of drugs, involving the exposure of bones in a patient not exposed to ionizing radiation in the head and neck area.[1] It is associated with significant local pain, the mobility or loss of the teeth, paresthesia, and the presence of intra- and/or extraoral fistulas with oozing purulent contents. Medication-related osteonecrosis of the jaw often leads to a significant reduction in the quality of life (QoL) and the disfigurement of the patient.[2]

The occurrence of MRONJ is associated with therapy with anti-resorptive drugs (bisphosphonates), receptor activator of nuclear factor-kappa B ligand (RANKL) drugs, e.g., denosumab, and anti-angiogenic drugs that are vascular endothelial growth factor (VEGF) inhibitors.[3,4]

According to the American Association of Oral and Maxillofacial Surgeons (AAOMS), the following criteria are used to diagnose MRONJ: current or past treatment with anti-resorptive or anti-angiogenic drugs; visible bone exposure, or the possibility of the intra- or extraoral probing of the jaw bones through a cutaneous or mucosal fistula for a period longer than 8 weeks; no history of radiotherapy to the jaw area; and no cancer metastases to this area.[4]

The AAOMS more specifically distinguishes 4 stages of MRONJ[4]:

– stage 0 – non-specific clinical symptoms, i.e., jaw pain and osteonecrosis, without the exposure of the jaw;
– stage I – exposed/necrotic bone in patients without symptoms of infection;
– stage II – exposed/necrotic bone associated with infection, presenting with pain and erythema in the area of the exposed bone, with or without a purulent exudate;
– stage III – exposed/necrotic bone in patients with pain, infection, and one or more of the following: a pathological fracture; an extraoral fistula; or osteolysis extending to the floor of the sinus.

At the molecular level, the mechanism of bone destruction in MRONJ is related to an imbalance in the RANK/RANKL/OPG (receptor activator of nuclear factor-kappa B/receptor activator of nuclear factor-kappa B ligand/osteoprotegerin) system. The ratio of RANKL to OPG plays an important role in initiating and maintaining osteoclastogenesis, and thus in regulating bone resorption, which is crucial for bone remodeling.[5] Factors that play a vital role in the pathomechanism of MRONJ include the inhibition of bone resorption and remodeling, the inhibition of angiogenesis, and vitamin D deficiency. Also immune system dysfunctions, e.g., those connected with diabetes mellitus (DM) linked to obesity, as well as genetic predispositions, might lead to MRONJ.[1,5] Mechanical and thermal injuries should also be taken into consideration.[6] Local, intraoral risk factors for MRONJ include areas of thinned oral mucosa over bone exostoses or an atrophic mandible, the presence of periodontal disease as additional local inflammation, and poor oral hygiene.[7–9]

The objective of this cohort observational study was to identify risk factors for the development of MRONJ.

Materials and methods

The medical records of patients hospitalized in the years 2015–2022 in the Department of Maxillofacial Surgery of the Ludwik Rydygier Specialist Hospital in Krakow and the Clinical Department of Maxillofacial Surgery of University Hospital in Krakow, Poland, were retrospectively analyzed. The study included patients treated for MRONJ in the maxilla and/or the mandible with a history of past bisphosphonate therapy. Patients with symptoms of osteonecrosis after radiotherapy of the head and neck region were excluded from the study.

The analyzed data was as follows: the patients' demographics (gender, age, the place of residence, education); comorbidities (anemia, DM, hypertension, other); the initial disease treated with bisphosphonates; the route of drug administration; the type of causative dental surgery; the area of necrosis; the MRONJ class according to the AAOMS criteria; and the bacterial cultures taken from the necrotized tissues. The analysis of data regarding the treatment used for MRONJ included surgical methods, pharmacotherapy, antibiotic therapy, anti-inflammatory drugs, and supportive treatment.

The relationship between the location of MRONJ and the place of the performed dental surgery, the relationship between the MRONJ stage and the route of administration of bisphosphonates, and the relationships between the MRONJ stage and parameters such as gender, age,

the place of residence, education, the nature of work, the initial reason for using drugs, the presence of comorbidities, and the performed dental procedure as a direct cause of MRONJ were examined.

Statistical analysis

Statistical analysis was performed using the R software, v. 4.2.1 (https://www.r-project.org). The analysis of qualitative variables (i.e., not expressed in numbers) was performed by calculating the number and percentage of occurrences of each value. The comparison of qualitative variables in groups was performed using the χ^2 test (with Yates correction for 2×2 tables) or Fisher's exact test, where low expected numbers appeared in the tables. A significance level of 0.05 was adopted in the analysis. Therefore, all *p*-values below 0.05 were interpreted as indicating significant relationships.

Results

A total of 43 patients hospitalized in both centers met the inclusion criteria for the study. Female subjects were predominant (n = 29; 67.4%), and the largest group were patients over 70 years of age (n = 22; 51.2%), living in cities with over 500,000 inhabitants (n = 16; 37.2%), with vocational education (n = 13; 30.2%). Detailed data is presented in Table 1.

The most common comorbidities in the studied group of patients were anemia (n = 34; 79.1%), DM (n = 17; 39.5%) and hypertension (n = 13; 30.2%).

Bisphosphonates were used in 30 patients (69.8%) in the course of cancer treatment (breast and prostate cancer), while 10 patients (23.3%) were treated for osteoporosis, 2 (4.7%) for osteopenia, and for one patient (2.3%) there was no data. It is also worth noting that male subjects were treated for osteoporosis much less frequently (9 subjects vs. 1 subject; 20.9% vs. 2.3%). The reasons for administering bisphosphonates by patient gender are shown in Fig. 1.

Bisphosphonates were most often administered intravenously – in 19 patients (44.2%). The oral route of administration was used in 11 patients (25.6%). The medical records of 13 patients (30.2%) did not include information on the route of drug administration.

In the studied group of patients, MRONJ was diagnosed in the mandible in 25 subjects (58.1%), and in the maxilla, in 18 cases (41.9%) (Table 2). The most frequently diagnosed stage of necrosis according to the AAMOS classification was stage 0 in 18 subjects (41.9%), followed by stage II in 16 subjects (37.2%). Detailed data is presented in Table 3.

Medication-related osteonecrosis of the jaw developed without an identifiable local cause in 29 cases (67.4%), in contrast to 14 patients (32.6%) whose necrosis was initiated by a dental procedure, most often tooth extraction.

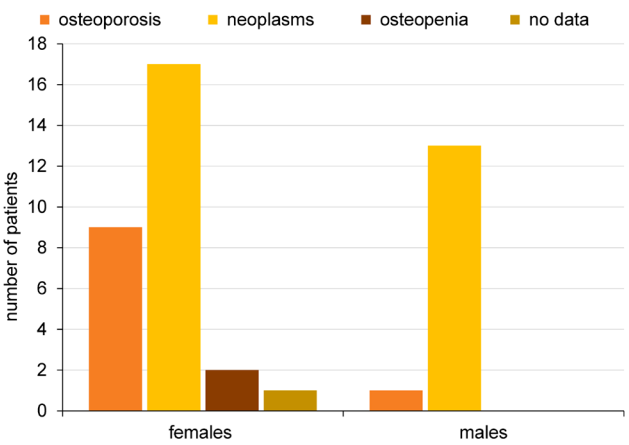

Fig. 1. Reasons for administering bisphosphonates in female and male subjects

Table 1. Demographic structure of the studied group of patients (N = 43)

Parameter		*n* (%)
Gender	F	29 (67.4)
	M	14 (32.6)
Age [years]	<60	6 (14.0)
	60–70	15 (34.9)
	>70	22 (51.2)
Place of residence	village	7 (16.3)
	city with up to 50 thousand inhabitants	10 (23.3)
	city with 50–500 thousand inhabitants	10 (23.3)
	city with over 500 thosand inhabitants	16 (37.2)
Education	primary	6 (14.0)
	vocational	13 (30.2)
	secondary	12 (27.9)
	higher	11 (25.6)
	no data	1 (2.3)

F – female; M – male.

Table 2. Incidence of medication-related osteonecrosis of the jaw (MRONJ) in the maxilla and the mandible

Location of MRONJ	After dental surgery	No identifiable local cause	Total
Maxilla	9 (20.9)	9 (20.9)	18 (41.9)
Mandible	5 (11.6)	20 (46.5)	25 (58.1)

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

Table 3. Staging of medication-related necrosis of the jaw (MRONJ) according to the American Association of Oral and Maxillofacial Surgeons (AAOMS) classification

Stage of MRONJ	*n* (%)
0	18 (41.9)
I	2 (4.7)
II	16 (37.2)
III	6 (14.0)
No data	1 (2.3)

Detailed data is presented in Table 4. Following dental procedures, necrotic lesions appeared at the surgical site. Detailed data regarding the location of MRONJ developing after dental surgery is presented in Table 5.

The patients underwent surgical and non-surgical treatment for MRONJ. The most common surgical methods were sequestrectomy in 28 cases (65.1%), and the segmental resection of the maxilla or the mandible in 17 patients (39.5%).

The results of microbiological tests conducted on the material collected from the patients showed the presence of Gram-positive bacteria, such as *Staphylococcus aureus* and *Streptococcus mitis*, as well as Gram-negative ones – *Klebisiella oxytoca*, *Escherichia coli* and *Pseudomonas aeruginosa*.

The most commonly identified bacterium was *Escherichia coli*, found mainly in male patients over 70 years old and females over 60 years old. The prevalence of bacteria highly adapted to anaerobic conditions was observed. The isolated microorganisms represented flora typical for healthcare-associated infections in our hospital environment.

Pharmacological treatment included antibiotic therapy, possibly targeted, and anti-inflammatory drugs. The most frequently used antibiotics and drugs included augumentin, amoksiklav, clindamycin, cipronex, and the chemotherapy drug metronidazole. The treatment process was also supported by hyperbaric oxygen therapy.

It was found that when bisphosphonates were administered orally, the most common type of necrosis was stage II, whereas in the case of intravenous administration it was stage 0. This relationship was statistically significant ($p = 0.048$) (Table 6). There were no statistically significant relationships between the MRONJ stage and parameters such as gender, age, the place of residence, education, the nature of work, the initial reason for using drugs, the presence of comorbidities, and the performed dental surgery as a direct cause of MRONJ. The stage of MRONJ did not depend on its location (the maxilla vs. the mandible).

Table 4. Causes of medication-related necrosis of the jaw (MRONJ)

Cause of MRONJ		*n* (%)
Drug treatment, with no identifiable local cause		29 (67.4)
Dental procedure	extraction	13 (30.2)
	periodontal treatment	1 (2.3)

Table 5. Relationship between the location of medication-related necrosis of the jaw (MRONJ) and prior dental surgery

Location of MRONJ caused by dental procedures	Place of the performed procedure	*n* (%)	*p*-value
Maxilla	lateral segment	7 (16.3)	<0.001*
	anterior segment	2 (4.7)	
Mandible	lateral segment	4 (9.3)	
	anterior segment	1 (2.3)	

*statistically significant.

Table 6. Relationship between the medication-related osteonecrosis of the jaw (MRONJ) stage and the route of administration of bisphosphonates

Stage of MRONJ	Route of administration of bisphosphonates			*p*-value
	oral ($n = 11$)	intravenous ($n = 19$)	no data ($n = 12$)**	
0 ($n = 18$)	3 (27.3)	11 (57.9)	4 (33.3)	0.048*
I ($n = 2$)	0 (0.0)	2 (10.5)	0 (0.0)	
II ($n = 16$)	8 (72.7))	3 (15.8)	5 (41.7)	
III ($n = 6$)	0 (0.0)	3 (15.8)	3 (25.0)	

Data presented as *n* (%).
*statistically significant; ** one chart contained no data on the progress of MRONJ.

Discussion

In the analyzed group of patients, MRONJ most often occurred in female subjects, which is consistent with the results of studies conducted by other authors.[10,11] According to Lira[12] and Jeong et al.,[13] MRONJ most often appears in the 7th decade of life. In our own material, the age of the patients was higher, and MRONJ was diagnosed mainly in the 8th decade of life.

In the study group, no statistically significant relationship between comorbidities and the incidence of MRONJ was observed, although anemia and DM were the most commonly encountered comorbidities. This discrepancy with regard to other studies, which stress the importance of comorbidities, might be caused by a relatively small sample size in our study. Further analysis of this issue is necessary. Magremanne et al. included hematological disorders (sickle cell anemia, β-thalassemia, coagulation disorders, etc.), metabolic disorders (DM, hypercholesterolemia, hyperlipidemia, Cushing's disease, etc.), rheumatological disorders (disseminated lupus erythematosus, rheumatoid arthritis, etc.), exogenous factors (alcohol, tobacco), and infectious factors (viral, bacterial, fungal) among the systemic factors affecting the development of MRONJ in patients taking bisphosphonates.[14] Gavaldá and Bagan also emphasized in their work the influence of comorbidities, such as DM, anemia or rheumatoid arthritis, and the patient's habits (smoking, drinking alcohol) on the occurrence of MRONJ.[15] Anastasilakis et al., stressed the importance of DM, rheumatoid arthritis, smoking, drinking alcohol, obesity, anemia, and HIV infection as systemic risk factors for MRONJ.[1]

In our study, bisphosphonates were administered mainly during the course of oncological (multiple myeloma, breast cancer and prostate cancer) treatment, which is consistent with the observations of other authors.[3,16–19] The remaining cases of bisphosphonate therapy referred to osteoporosis and osteopenia.

In their retrospective work, Farrugia et al. analyzed the impact of new-generation bisphosphonates – zolendronate, pamidronate and alendronate – on the development of MRONJ.[20] Of the 23 patients diagnosed with MRONJ, 18 subjects were treated with the intravenous form

of a drug.[20] Lira also noted in his paper that the highest percentage of MRONJ occurred as a result of the intravenous use of bisphosphonates.[12] Gavaldá and Bagan included the route and duration of drug administration during the course of the underlying disease as systemic risk factors for the development of MRONJ.[15] Similar results were obtained in the present study. It is noteworthy that with the intravenous administration of bisphosphonates, type I necrosis developed statistically significantly more often. No similar relationship was found in the available literature.

Our research showed a slightly higher incidence of MRONJ in the mandible than in the maxilla. This result is consistent with those obtained by Migliorati et al.,[3] Bamias et al.[16] and Saad et al.[21] Bamias et al. found that in a studied group of 17 subjects, necrosis developed in the mandible in 14 cases and in the maxilla – in 3 cases.[16]

Patients at dental offices are at increased risk of developing medication-related necrosis. It has been shown that MRONJ may be a consequence of a previously performed dental procedure, e.g. tooth extraction, implant surgery, a periodontal procedure, endodontic treatment, trauma caused by a denture plate, or a hygienization procedure.[1] Research conducted in Sweden by Hallmer et al. on the causes of MRONJ indicates that the most common factor predisposing to the development of osteonecrosis is tooth extraction, followed by a periodontal procedure and an injury caused by a denture plate.[22,23] Similarly, Lira[12] and McGowan et al.[24] identified tooth extraction as the main factor causing MRONJ. This is consistent with the results of our own research.

In the available reports, the incidence of spontaneous necrosis varies from 2.2% to 60%.[25,26] Although the definition of spontaneous MRONJ is fairly clear (no previous dental problems, injury or therapy), it is possible that in some cases, the patients are not aware of painless, necrotic, exposed bone.[27] Among our patients, a high percentage (67.4%) of spontaneous necrosis was observed, which differs from the results presented by other authors.[20,23] In our opinion, that may have been caused by micro-injuries in the oral cavity, previously unnoticed by the patients.

Current knowledge about the treatment of MRONJ is presented in a diagram prepared by the authors (Fig. 2). Surgical treatment is preferred; Vassiliou et al. recommend such treatment in stages III and IV of necrosis, while implementing conservative treatment in stages I and II.[28] Other methods include laser surgery, which supports the repair process by stimulating the growth of lymphatic and blood vessels.[16] Hyperbaric oxygen therapy and ozone therapy are also used in complementary treatment.[17,29] In our study, all patients with MRONJ underwent surgical treatment, regardless of the stage of necrosis.

Hallmer's research on the bacterial flora of necrotic bone in the course of MRONJ showed the presence of *Porphyromonas* spp., *Lactobacillus* spp., *Tonarella* spp., *Prevotella* spp., *Actinomyces* spp., *Treponema* spp., *Streptococcus* spp., and *Fusobacterium* spp.[22] In turn,

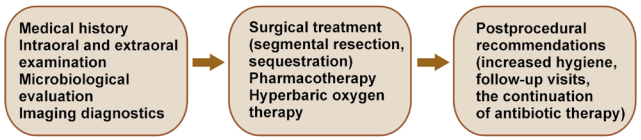

Fig. 2. Treatment regimen in the group of patients described in the article

Zirk et al.,[18] Cerrato et al.[30] and Micheletti et al.[31] identified biofilms containing *Streptococcus* spp., *Prevotella* spp., *Actinomyces* spp., *Veillonella* spp., and *Parvimonas micra* in the cultures from the tissues collected from patients with MRONJ. Ewald et al. showed the presence of *Actinomyces* spp., *E. coli*, *Veionella parvula*, *Enterobacter* spp., *Lactobacillus* spp., *Neiseria* spp., *Enterococcus* spp., *Eikenella* spp., and *Fusobacterium* spp.[32]

Our microbiological evaluations of the necrotic tissue in the patients with MRONJ revealed the presence of bacteria typical for hospital-acquired infections, such as *E. coli* and *P. aeruginosa*. That was probably related to the prolonged therapy, multiple hospitalizations and immunological condition of the patients, especially those receiving oncological treatment.

The obvious limitations of our study are a small sample size, as well as the retrospective character of this research. These factors limit the generalizability of our findings.

Conclusions

Risk factors for the development of MRONJ in patients treated with bisphosphonates include the intravenous route of drug administration, past intraoral surgery, female gender, and senior age

The prevention of MRONJ requires the education of both patients and the dentists performing basic intraoral procedures. The decision to start therapy with bisphosphonates should also be preceded by a thorough examination of the oral cavity and the elimination of potential foci that may lead to the development of osteonecrosis of the jaw in the future.

Ethics approval and consent to participate

The study received approval from the Bioethics Committee of the Jagiellonian University, Krakow, Poland (approval No.: 1072.6120.33.2022).

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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Original paper

Impact of long-term stress on awake bruxism: An observational longitudinal within-subject study of stress-related changes

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Abstract

Background. Awake bruxism (AB), characterized by repetitive jaw muscle activity during wakefulness, is often associated with stress, anxiety and depression.

Objectives. The aim of the study was to examine the long-term relationships between psychological distress, resilience and AB behaviors during stressful periods. To this end, a longitudinal, within-subject design of the study was used.

Material and methods. A repeated-measures design was employed to evaluate 136 individuals. The participants underwent 2 assessments: the baseline evaluation conducted at the onset of an armed conflict (phase 1); and the follow-up evaluation, performed 1 year later, when the conflict remained ongoing (phase 2). Each subject served as their own control. At each phase of the study, the participants completed a self-report questionnaire, the Oral Behavior Checklist (OBC), which addressed self-awareness of performance of teeth grinding, teeth clenching, tooth contact, and/or mandible bracing while awake. A score of 2 and above on any of the questions indicated positive awareness of the presence of AB behaviors. The additional questionnaires referred to subjects' ability to cope with stress adaptively, their ability to recover from stress, perceived stress, screening for depressive and anxiety symptoms, and screening for adjustment disorder and post-traumatic stress disorder (PTSD).

Results. A significant increase in teeth clenching was apparent during phase 2. The subjects' ability to cope with stress in an adaptive manner, as well as their perceived stress levels, increased the likelihood of AB behaviors during both phases (odds ratios ranging from 11% to 27%).

Conclusions. Awake bruxism behaviors in general, and teeth clenching in particular, should be considered as possible stress-relieving behaviors.

Keywords: coping, resilience, psychological distress, awake bruxism, AB

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Highlights

- During extended periods of stress, higher perceived stress and reliance on adaptive coping strategies were associated with increased awake bruxism (AB) behaviors.
- Awake bruxism behaviors, particularly teeth clenching, may be linked to stress-coping mechanisms.
- Given the harmful effects of teeth clenching, individuals using this behavior as a coping strategy should receive preventive care and regular monitoring.

Introduction

Awake bruxism (AB) is defined as repetitive jaw muscle activity during wakefulness, characterized by continuous or recurrent tooth contact and/or bracing or thrusting of the mandible.[1] Studies on AB have transitioned from perceiving bruxism as merely a pathological condition to recognizing it as physiological masticatory muscle activity. Bruxism has the potential to act as a risk factor for certain medical conditions, but it can also serve as a protective factor.[2,3] A recent update to the 2018 consensus on bruxism assessment provided a glossary of all constituent terms used in the definitions of unspecified, sleep and awake bruxism that have been published previously.[4] With a global prevalence of 16–32%, AB represents a significant concern for healthcare professionals.[5] Tooth contact is considered to be the most prevalent AB behavior.[6,7]

Awake bruxism is influenced by psychological, biological and genetic factors.[8,9] Psychological factors associated with AB include personality traits, such as neuroticism and somatization,[10] anxiety, depression,[11] and stress sensitivity.[12] Widespread stress can be attributed to various sources, including the coronavirus disease 2019 (COVID-19) pandemic, natural disasters and war. During the pandemic, there was a notable increase in the prevalence of bruxism and temporomandibular symptoms. The impact of the pandemic on temporomandibular disorders (TMD) and AB was more extensive than initially expected.[13,14]

The physiological response to stress is primarily mediated by the hypothalamic–pituitary–adrenal (HPA) axis, which serves as the body's central stress response system. Chronic stress can lead to the dysregulation of the HPA axis, characterized by prolonged activation of the HPA axis, which in turn disrupts cortisol regulation, leading to a decline in both physical and mental health.[15] The neural substrates underlying chronic stress responses may differ markedly from those involved in acute stress reactions, often engaging distinct limbic, hypothalamic and brainstem circuits. An individual's response to stress, whether acute or chronic, is shaped by a constellation of factors, including genetic predisposition, early life experiences, environmental influences, biological sex, and age. The contextual framework within which stressors are encountered plays an important role in determining whether the resultant stress responses are adaptive or maladaptive.[16] A meta-analysis performed by Chemelo et al. revealed a significant association between stress and bruxism.[17] However, the authors emphasized that the quality and reliability of the evidence are low, and signaled the necessity for additional studies to understand this relationship more comprehensively.[17]

Recent research suggests that AB may play a positive role in stress coping. This finding is compatible with the hypothesis of mastication as a means of relieving psychological tension,[10] which indicates that bruxism might serve as an adaptive mechanism for managing stress, rather than simply being a pathological consequence.

War, one of the most intense stressors, significantly affects mental health by inducing severe stress and anxiety, even among individuals not directly involved in combat. Since the terrorist attack on Israel on October 7, 2023, the notion that the nation was experiencing national trauma was suggested. This trauma was believed to cause long-lasting implications for the future.[18]

Initial results indicated that within the first 3 months of the armed conflict, 68% of the participants were identified as having adjustment disorder, a maladaptive response to stressors that typically occurs within 3–6 months of the stressful stimulus.[19] Subjects with adjustment disorder exhibited less muscle relaxation and more teeth clenching and grinding than those without the disorder. The armed conflict has been continuing for over a year, leading to an increased incidence of more severe stress-related conditions, such as post-traumatic stress disorder (PTSD). Several studies have documented the escalating prevalence of PTSD among the Israeli civilian population following October 7, 2023.[20–22]

To date, there is a limited understanding regarding how the same individuals respond to prolonged stress regarding AB.[17] Such understanding can be achieved through longitudinal studies examining AB under prolonged stress conditions. The ongoing conflict in Israel creates conditions for studying the impact of prolonged, population-wide stress on AB behavior. A previous study compared 2 different groups of subjects during different periods of time (one group was examined during peaceful time and the other during the beginning of the armed

conflict).[19] The present study adopts a different approach and attempts to evaluate the same group of subjects twice (a within-subject design), during the armed conflict. Specifically, the objective was to longitudinally monitor subjects' AB behavior from the initial months of the armed conflict through a one-year period, during which the conflict remained active. Additionally, the aim of this study was to examine the long-term relationships between psychological distress, resilience and AB behavior.

The hypothesis of the study posits that there will be an increase in AB behaviors between phase 1 and phase 2. This increase will be mediated by stress, anxiety, depression, and resilience.

Material and methods

Population

To investigate intraindividual change, the study utilized a repeated-measures design, evaluating the same cohort of participants at 2 distinct time points across a 12-month interval. The study population comprised a convenience sample of dental students aged >18 years from the Maurice and Gabriela Goldschleger School of Dental Medicine at Tel Aviv University, Israel, the largest academic institution in the country. The university's student body includes individuals from diverse ethnic, cultural and social backgrounds, including Jewish, Muslim, Christian, secular, and Orthodox groups.

The data collection was conducted independently by 3 dental students (AYC, NV, MZ) to minimize researcher bias. Participants were approached twice during 2 different time periods (phase 1 and phase 2). Each subject served as their own control. No financial compensation was provided for participation. The exclusion criteria were diagnosed neuromuscular and/or joint diseases, confirmed depression, and trauma to the head or jaw during the past 6 months.

The study received ethical approval from Tel Aviv University (approval No. 0009558-2). Written informed consent was obtained from all participants.

Phase 1

The data was collected in January 2024. This period has been marked by significant stress, extending over a duration of 3 months following the terrorist attack that occurred on October 7, 2023, and persisting throughout the subsequent armed conflict and massive missile attacks on the Tel Aviv area.

Phase 2

Participants who were assessed in phase 1 were reapproached 1 year later, in January 2025. The ongoing armed conflict and the continuous missile attacks on civilian population centers (occurring both during daytime and night) led to prolonged exposure to stress, which resulted in high levels of uncertainty, insecurity and instability.

Tools

The following questionnaires in Hebrew were administered to the participants via Google Forms links:

– self-report regarding the performance of 4 AB behaviors (teeth grinding, teeth clenching, tooth contact, and/or mandible bracing) while awake, based on the last month.[23–26] The questions were part of the Oral Behavior Checklist (OBC). The validity of the OBC was proven in several studies.[27,28] The version used in the present study is part of the official Hebrew version of the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) Axis II.[29,30] The response scores range from 0 (none of the time) to 4 (all of the time). A score of 2 and above on any of the questions indicated positive awareness of the presence of AB behaviors;
– Brief Resilience Coping Scale (BRCS), which is a component of the Standardized Tool for the Assessment of Bruxism (STAB).[31] The BRCS evaluates an individual's ability to cope with stress adaptively, emphasizing their capacity to use coping strategies with flexibility and persistence to tackle problems, even when faced with stressful situations. The participants were asked to rate how well specific statements described their behaviors or actions using a scale from 1 (does not describe me at all) to 5 (describes me very well). The BRCS is unidimensional, representing 1 latent factor.[32] The total score, ranging from 4 to 20, can be categorized into low (4–13), medium (14–16) and high (17–20) resilience coping[33];
– Brief Resilience Scale (BRS), which evaluates the ability to recover from stress. It comprises 6 statements, rated on a scale from 1 (strongly disagree) to 5 (strongly agree). The total score ranges from 6 to 30, and when divided by 6, can be categorized as low resilience (≤2.9) or normal/high resilience (≥3.0)[34];
– Patient Health Questionnaire-4 (PHQ-4), which is part of the official Hebrew version of the DC/TMD Axis II.[20] The PHQ-4 is a reliable and valid tool for screening depressive and anxiety symptoms in both clinical and non-clinical populations.[35] The total score ranges from 0 to 12, and is typically assessed using the following cut-off scores: normal (0–2); mild distress (3–5); moderate distress (6–8); and severe distress (9–12);
– Perceived Stress Scale-10 (PSS-10), which is used worldwide, and the validity and reliability of which have been proven in numerous studies.[36] An official Hebrew version of the questionnaire was used.[37] The questionnaire measures the frequency of stress experienced over the past month through 10 items, with

responses ranging from 0 (never) to 4 (very often). The total score ranges from 0 to 40 and can be categorized as low stress (0–13), moderate stress (14–26) and high stress (27–40)[38];
- ultra-brief version of the Adjustment Disorder New Module (ADNM-4), which serves as a brief screening tool to assess symptoms of adjustment disorder.[39] The participants were asked to indicate the frequency of items on a 4-point Likert scale, ranging from "never" to "often". A score of 8.5 or higher is recommended for the diagnosis of subjects with adjustment disorder[40];
- Primary Care PTSD Screen for Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (PC-PTSD-S), is a validated screening tool designed to identify individuals in primary care who may have probable PTSD. It was collected during phase 2 only, and it aligns with the diagnostic criteria from the DSM-5. The PC-PTSD-5 is a 5-item screening tool designed to identify individuals with probable PTSD.[41] The responses are scored on a scale from 0 to 5. A cut-off point of 4 ideally balanced false negatives and false positives for the overall sample and for men. However, for women, a cut-off point of 4 resulted in a high number of false negatives. In some cases, a lower cut-off point is considered, as performance parameters may vary depending on the sample.[42]

Statistical analysis

The sample size calculation, based on a medium effect size of $d = 0.5$, a statistical power of 80%, a two-tailed significance level of $\alpha = 0.05$, and an assumed correlation between measurements of 0.5, required the collection of responses in both examination periods from a minimum of 27 participants.

Multiple Wilcoxon signed-rank tests were used to compare psychological and behavioral variables between phases. Logistic regression analyses were performed to determine which psychological self-report measures showed the ability to predict subjects' awareness of AB behaviors at each of the time phases.

To assess the robustness of the findings, a series of sensitivity analyses were performed. First, the behavioral and psychological outcome variables were re-evaluated using paired *t*-tests alongside the primary Wilcoxon signed-rank test to verify the consistency of results under parametric assumptions. Second, to guard against false discovery, the Benjamini–Hochberg correction, at a false discovery rate (FDR) of 0.05, was applied. Third, the internal consistency of psychological scales was verified using Cronbach's alpha. Finally, the estimation of the robust effect size was calculated using Cohen's *d*.

The statistical analyses were performed using the IBM SPSS Statistics for Windows software, v. 29 (IBM Corp., Armonk, USA) and Jamovi v. 2.6 (https://www.jamovi.org/download.html).

Results

Population

During the 1st phase, 150 students completed the self-report questionnaires. Of the initial group, 136 subjects consented to participate in the follow-up evaluation during the 2nd phase (91% response rate, 62.5% female, mean age: 28.4 ±3.7 years). The data indicates that 46% of the subjects were single, while the remaining subjects were either married or living with a spouse. The mean number of children per participant was 0.3, with the mean age of the youngest child being 2.16 ±2.15 years. The reasons for not participating in the 2nd phase were absence at the time of data collection (sick leave, military service recruitment, etc.) and/or reluctance to participate due to lack of interest.

Oral behaviors

A large majority of the participants demonstrated awareness of AB behaviors in both phases (67.6% and 75.7%, respectively). The difference between the phases did show statistical significance.

To examine changes in different AB behaviors from the 1st to the 2nd phase, the Wilcoxon signed-rank test was performed. The analysis revealed no missing values in either variable. Initial analyses indicated a statistically significant increase in clenching behavior ($Z = -2.361$, $p = 0.018$), with no significant changes in grinding ($p = 0.372$), tooth contact ($p = 0.424$) or mandible bracing ($p = 0.107$). Following the Benjamini–Hochberg correction, only clenching remained significant (Table 1, Fig. 1). The paired *t*-tests largely confirmed the findings from the Wilcoxon test, thereby increasing confidence in the results.

Self-report psychological measures

The reliability of self-report psychological measures (Chronbach's alpha) was as follows (phase 1 and 2, respectively): PHQ-4 – 0.85, 0.87; ADNM-4 – 0.82, 0.81; BRCS – 0.70, 0.66; BRS – 0.73, 0.77; PSS-10 – 0.89, 0.91; PC-PTSD-S (2nd phase only) – 0.67.

In general, subjects in both phases reported moderate stress levels (PSS-10), presented mild distress levels (PHQ-4), demonstrated medium ability to cope with stress adaptively (BRCS), and displayed normal or high ability to recover from stress (BRS).

To evaluate the differences between the 1st and 2nd phases across the 5 psychosocial measures, the Wilcoxon signed-rank test was used. The analysis revealed no missing values in either variable. The raw *p*-values indicated a significant change for ADNM-4 ($p < 0.001$), BRCS ($p = 0.047$) and PSS-10 ($p = 0.003$), while other measures did not reach the level of statistical significance

Table 1. Comparison of awake bruxism (AB) behaviors between the 1st and 2nd phases of the study ($N = 136$)

Behavior	Phase 1	Phase 2	*p*-value	BH threshold	Effect size (Cohen's *d*)
Grinding *M* ±*SD*	0.61 ±0.92	0.71 ±0.94	0.372	0.03	0.08
Clenching *M* ±*SD*	1.41 ±1.06	1.71 ±1.00	0.018*	0.01	0.20
Tooth contact *M* ±*SD*	1.60 ±1.12	1.69 ±1.04	0.424	0.05	0.07
Mandible bracing *M* ±*SD*	1.29 ±1.19	1.50 ±1.15	0.107	0.02	0.14

* statistically significant ($p < 0.05$, Wilcoxon signed-rank test); *M* – mean; *SD* – standard deviation; BH – Benjamini–Hochberg. Each behavior was evaluated on a scale ranging from 0 to 4.

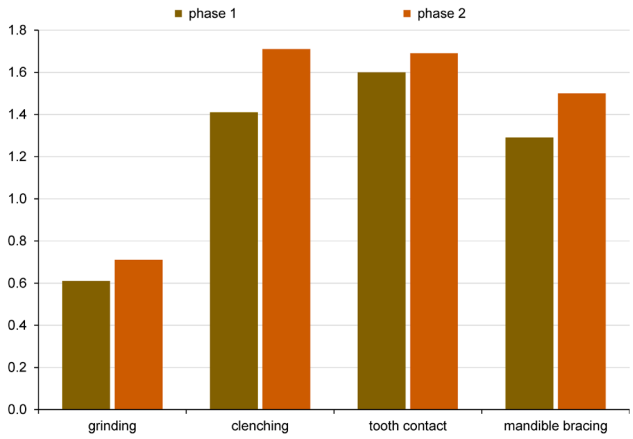

Fig. 1. Variations in awake bruxism (AB) behaviors across 2 time phases
Each behavior was evaluated on a scale ranging from 0 to 4.

(all $p > 0.05$). Following the Benjamini–Hochberg correction, only ADNM-4 and PSS-10 retained statistical significance (Table 2). The paired *t*-tests largely confirmed the findings of the Wilcoxon test, thereby increasing confidence in the results.

The study revealed no significant differences in AB behaviors between participants with high scores (≥4) on the PC-PTSD-S (78.6% reporting AB behaviors) and those who attained scores below 4 (75.4% reporting AB behaviors), with only a 3.2 percentage point difference (2nd phase). The results remained similar when a cut-off point of 3 was applied (78.4% vs. 75.4%, respectively).

Multivariate logistic regression

To determine which psychological self-report measures could predict participants' awareness of AB behaviors, logistic regression analyses were conducted for each phase. Initially, each variable was evaluated through a series of binomial logistic regression models. The variables that demonstrated significant predictive ability were incorporated into the multiple logistic regression models (Table 3).

In both the 1st and 2nd phases, an increase in BRCS was associated with a higher probability of AB behaviors (rise by 26% and 27%, respectively). An increase in PSS-10 led to an 11% and a 16% rise, respectively, in the odds of AB behaviors.

In the 1st phase, the model explained 14% of the variance in the dependent variable (Nagelkerke's $R^2 = 0.14$). In the 2nd phase, the model explained 21% of the variance in the dependent variable (Nagelkerke's $R^2 = 0.21$).

Table 2. Comparison of self-report psychological measures between the 1st and 2nd phases of the study ($N = 136$)

Measure	Phase 1	Phase 2	*p*-value	BH threshold	Effect size (Cohen's *d*)
ADNM-4 *M* ±*SD*	9.28 ±2.71	7.62 ±2.49	<0.001*	0.01	0.47
BRCS *M* ±*SD*	14.90 ±2.51	15.42 ±2.35	0.047*	0.03	0.17
BRS *M* ±*SD*	19.88 ±3.79	19.41 ±1.94	0.154	0.04	0.12
PSS-10 *M* ±*SD*	18.08 ±6.39	15.95 ±6.88	0.003*	0.02	0.25
PHQ-4 *M* ±*SD*	4.08 ±2.95	3.76 ±2.93	0.344	0.05	0.08

* statistically significant ($p < 0.05$, Wilcoxon signed-rank test); ADNM-4 – Adjustment Disorder New Module; BRCS – Brief Resilience Coping Scale; BRS – Brief Resilience Scale; PSS-10 – Perceived Stress Scale-10; PHQ-4 – Patient Health Questionnaire-4.

Table 3. Predicted likelihood of AB behaviors reported by the study subjects in the 1st and 2nd phases of the study

Phase	Predictor	Estimate	*SE*	*Z*	*p*-value	*OR*	95% *CI*	
							lower	upper
Phase 1	BRCS	0.2305	0.0857	2.691	0.007*	1.26	1.06	1.49
	PSS-10	0.1060	0.0392	2.707	0.007*	1.11	1.03	1.20
Phase 2	BRCS	0.2398	0.1029	2.33	0.020*	1.27	1.03	1.56
	PSS-10	0.1494	0.0403	3.711	<0.001*	1.16	1.07	1.26

* statistically significant ($p < 0.05$, Wilcoxon signed-rank test); *SE* – standard error; *OR* – odds ratio; *CI* – confidence interval. The estimates represent the log odds of AB behaviors = yes vs. AB behaviors = no at the 1st and 2nd phases. The models were adjusted for age and sex.

Discussion

Bruxism remains a considerable diagnostic and therapeutic challenge due to its complexity and incomplete understanding of its pathophysiology. This persistent uncertainty underscores the necessity for the continuous exploration of novel therapeutic strategies.[43] For example, a study that examined the effect of TMD treatment on self-reported sleep bruxism (SB) and AB found that subjects who received a combination of counseling and other treatments exhibited an increase in the frequency of AB bracing, potentially attributable to increased awareness of the condition.[44]

The present study employed a longitudinal design in a real-life stress context, namely an armed conflict. Although the associations between stress and AB have been demonstrated previously, longitudinal follow-up of subjects over a period of time enables the observation of how variables change within individuals under stressful conditions. Given that the study participants are the same individuals, any differences between subjects are minimized. This methodological approach enables the focus on temporal changes. Longitudinal follow-up studies on AB are scarce, although some recent research has attempted to address this issue.[45]

The current study examines a gap in our understanding of AB behaviors, namely how individual AB patterns and associated psychological aspects change when exposed to prolonged stress. The study examines a real-world scenario of shared, prolonged stress exposure among all participants.

The second phase of the study revealed that teeth clenching was the only behavior to exhibit a statistically significant increase. Of the 4 studied AB behaviors (tooth contact, mandible bracing, teeth clenching, and teeth grinding), only 2 present repeated masticatory muscle activity and forceful tooth contact (teeth clenching and teeth grinding). Unlike teeth grinding, which is rarely reported during waking hours (frequency ranging from 0.1% to 1.0%, based on the designated Ecological Momentary Assessment (EMA) approach), teeth clenching is markedly more frequent (frequency ranging between 2.0% and 11.2%).[46] Physiologically, teeth clenching activates a neural pathway within the sympathetic adrenomedullary axis, which leads to the release of norepinephrine. Norepinephrine plays a crucial role in physiological stress responses, potentially counteracting the activation of the HPA axis.[12] Under conditions of sustained stress, teeth clenching may constitute a protective behavioral adaptation that assists in the regulation of stress response.

Despite the extended conflict and ongoing physical threats to the population, the participants reported only moderate perceived stress levels (PSS-10), which further decreased during the second evaluation phase. During periods of prolonged stress, individuals may become psychologically habituated to stressors, yet their physiological response systems may remain sensitized, maintaining the relationship between bruxism and stress.[47] The moderate perception of stress and mild distress levels in the present study may be a result of the subjects' ability to recover from stress (normal to high scores, as measured by the BRS). Israeli society, constantly exposed to security tensions and alerts, appears to be relatively resilient and demonstrates high stress recovery capabilities.[48]

The decrease in the prevalence of adjustment disorder during the second phase is not unexpected. The disorder involves a maladaptive response to stressors that typically occur within 3 months and resolve within 6 months. The data for the first phase was collected 3 months after October 7, 2023, during extremely stressful events, while the data for the second phase was collected a year later, when adjustment disorder might have been either resolved or transformed into more severe PTSD.

Post-traumatic stress disorder is a mental health condition triggered by the exposure to a traumatic event, such as war. Subjects diagnosed with PTSD have a higher prevalence of TMD diagnoses compared to controls.[49–51] Several studies have noted an increasing occurrence of PTSD among the Israeli civilian population following October 7, 2023.[21,22] In the present study, subjects who scored highly on the PC-PTSD-S (cut-off points of ≥3 or ≥4), as well as subjects with scores below the cut-off point, reported a high prevalence of AB behavior (>75%). This observation referred to the 4 studied behaviors (teeth grinding, teeth clenching, tooth contact, and/or mandible bracing). In the present study, reports were collected within

15 months of the ongoing conflict, a timeframe that may account for the elevated prevalence of AB behaviors. This may partially explain the lack of significant differences between PTSD-positive and PTSD-negative groups. Another possibility is that AB behaviors and PTSD may constitute distinct and independent responses to stress, rather than being causally linked.

The management of stress is highly influenced by individual coping strategies. Saczuk et al. showed that subjects with SB use maladaptive coping strategies more frequently than subjects without SB.[52] Soto-Goñi et al. demonstrated that awake bruxers display more adaptive coping strategies than subjects who do not manifest bruxism symptoms.[10] Although the BRS and BRCS are conceptually linked in measuring aspects of resilience, these instruments assess distinct phases within the adaptive response to stress. The inclusion of both scales enables the examination of whether recovery capacity and adaptive coping processes exhibit differential relationships with AB behaviors, thereby providing a more nuanced understanding of resilience mechanisms. The BRCS has been developed to assess individuals' propensity to adopt adaptive coping strategies in response to stress.[33] This scale evaluates individuals' ability to adapt to and manage challenging situations, in contrast to the BRS, which focuses on resilience as the capacity to recover from stress.[34] The overlap between the two reflects the complex and multifaceted nature of resilience.

The findings of the present study suggest that both perceived stress and resilient coping are associated with subjects' AB behaviors. Perceived stress has been identified as a significant contributing factor to the occurrence of AB and its clinical manifestations.[53] Popescu et al. demonstrated a meaningful correlation between stress and AB, recommending that stress be incorporated as an essential factor in the evaluation of bruxism.[54] These results are consistent with our findings and support including stress-related measures in the STAB tool.

While the capacity for stress recovery, as measured by the BRS, demonstrated clear effects, the influence of resilient coping strategies (BRCS) and the manifestation of depressive and anxiety symptoms (PHQ-4) appeared comparatively less pronounced. As previously indicated, AB behaviors in general, and teeth clenching in particular, may play a positive role in stress coping. Specifically, mastication serves as a means of managing psychological tension.[10] In a literature review, Kubo et al. claimed that chewing is a useful stress coping mechanism, because it alters the functions of the HPA axis and the autonomic nervous system.[55] Furthermore, it can minimize stress-induced changes in the hippocampus and hypothalamus.[55] Maciejewska-Szaniec et al. demonstrated that sequence variants in genes related to stress coping may be correlated with AB susceptibility via an elevated perceived stress level.[53] A recent review suggested that stress, as an initiating factor, increases muscle tone, and when this increase rises to 10–20%, it may cause a bruxism event, in addition to reducing the pain threshold.[12]

The present findings indicate that both perceived stress and resilience coping increase the likelihood of AB behaviors. The finding that subjects' tendencies to cope with stress in a highly adaptive manner (BRCS) increase the odds of AB seems paradoxical, as it suggests that AB behaviors may be part of the body's stress regulatory system, possibly providing proprioceptive feedback that contributes to the modulation of stress responses.[55] Awake bruxism behaviors serve as a form of adaptive, albeit unconscious, behavior that may help individuals maintain functionality during stressful periods and/or may act as a compensatory mechanism for inadequate psychological coping resources, acting as a physical outlet when cognitive coping strategies are insufficient.[56] Alternatively, subjects under stress may be more conscious of their AB behaviors, which could result in over-reporting.

Unfortunately, numerous parts of the world are afflicted by armed conflicts (e.g., Ukraine, Colombia, Africa). The impact of prolonged stress caused by wars on AB behaviors may affect the lives of millions. Individuals diagnosed with bruxism exhibit a high prevalence of TMD, with global co-occurrence rates estimated at approximately 17%, though these figures vary across different regions.[57] Recently proposed shortened screening tools for TMD and bruxism enable a better assessment of these conditions by general dentists.[58] Enhanced awareness of AB behaviors, alongside the implementation of lifestyle-based therapeutic interventions such as modifications to daily routines, sleep hygiene and dietary habits, may offer general benefits,[43] particularly for individuals exposed to sustained stress.

Limitations

The sample of our study consisted of dental students from a single academic institution. Even though the student body in the Dental School is quite diverse, including individuals from different ethnic, cultural and social backgrounds, the highly specific cohort may limit the generalizability of the findings. Regrettably, no information was collected regarding factors such as smoking and/or sleep quality. Moreover, AB behaviors were assessed through single-point self-report and not as a combination of single-point self-report with the EMA approach, as recommended by the recently introduced STAB.[31] While the single-point self-report method represents a valid and accessible means for evaluating bruxism behaviors, the EMA approach offers a more detailed real-time report on AB behaviors.[59] The self-report assessment of AB may be subject to recall and over-reporting bias. The longitudinal follow-up design, in which each subject serves as his or her own reference for comparison, presents some advantages, but further longitudinal studies using more accurate tools to define AB (e.g., electromyography) are necessary to explore these issues more thoroughly.

Clinical importance

Personal stress management techniques serve as essential coping mechanisms when facing prolonged adversity. Awake bruxism behaviors in general, and teeth clenching in particular, may play a positive role in stress coping and serve as a means of managing psychological tension.[10] As teeth clenching involves forceful occlusal forces, it may lead to adverse results such as abfractions and tooth fractures.[1,60] Accordingly, subjects who engage in such stress coping behaviors should be provided with preventive interventions and subjected to ongoing clinical monitoring to mitigate long-term health consequences.[61]

Conclusions

Perceived stress and an individual's capacity to use coping strategies with flexibility and persistence in addressing problems during stressful situations increase the likelihood of exhibiting AB behaviors over extended periods. The results suggest that AB behaviors in general, and teeth clenching in particular, may play a role in stress coping.

Ethics approval and consent to participate

The study received ethical approval from Tel Aviv University in Israel (approval No. 0009558-2). 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

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Sleep bruxism and sleep architecture in chronic migraine: A polysomnographic study

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Abstract

Background. Despite the fact that sleep bruxism (SB) is common in migraine, and that patients with migraine often report poor sleep quality, SB and sleep architecture in chronic migraine (CM) have not been fully explored.

Objectives. The aim of the study was to establish the association between SB and CM, with an assessment of sleep structure alterations in CM.

Material and methods. The diagnosis of migraine was made using the 3rd edition of the International Classification of Headache Disorders (ICHD-3). Sleep bruxism and sleep structure were assessed using polysomnography, according to the American Academy of Sleep Medicine (AASM) Guidelines. All results were adjusted for medication use in the treatment of migraine, which may interfere with sleep and SB.

Results. A total of 110 patients with migraine (mean age: 39.3 years; 88% female) were evaluated, including 65 individuals with CM and 45 episodic migraine (EM) patients. The patients with CM had lower REM sleep duration when compared to those with EM (median (*Me*): 21.4% of total sleep time (TST) vs. 24.4% of TST, $p = 0.008$), while REM sleep below 23.1% of TST was associated with increased odds of CM (odds ratio (*OR*): 3.61 (95% confidence interval (*CI*): 1.60; 8.15), $p = 0.002$). Seventy-six out of 110 (69%) participants were diagnosed with SB. The presence of mixed bruxism at a frequency of above 0.4 episodes per hour (n/h) was associated with increased odds of CM (*OR*: 2.40 (95% *CI*: 1.06; 5.46), $p = 0.048$). However, severe SB (bruxism episode index (BEI) >4) was associated with increased odds of migraine with aura (MwA) (*OR*: 2.68 (95% *CI*: 1.05; 6.83), $p = 0.044$). Migraine without aura showed a weak, negative correlation with BEI ($r = -0.293$, $p = 0.002$).

Conclusions. A decrease in the REM stage of sleep was associated with CM. Despite the high prevalence of SB in patients with migraine, SB was not associated with CM, while severe bruxism was associated with MwA. Therefore, if any association between SB and migraine exists, it is more likely related to aura phenomena than to migraine chronification.

Keywords: sleep, pain, bruxism, headache, chronification

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Highlights

- Patients with chronic migraine exhibit altered sleep structure, characterized by a decrease in the REM stage of sleep.
- Sleep bruxism is a frequent sleep disturbance observed in individuals with migraine.
- Sleep bruxism is not associated with chronic migraine.
- Aura phenomena in migraine may be associated with sleep bruxism.

Introduction

Primary headaches are characterized by the absence of underlying causes, such as injury, disease or pathological processes. These headaches are further classified into migraine, tension-type and cluster headaches.[1,2] Migraine is the third most disabling neurological disorder and affects 15.2% of the general population, mainly young females.[3] Due to the frequency of attacks, migraine can be classified as episodic (EM) or chronic (CM). In CM, headache attacks are present at least 15 days a month, with a minimum of 8 days of migraine.[4,5] Chronic migraine is considered more severe and is associated with increased disability, depressive symptoms and the presence of sleep disturbances.[4,6–10]

Sleep bruxism (SB) is defined as rhythmic (phasic) or non-rhythmic (tonic) masticatory muscle activity during sleep.[11] Sleep bruxism affects 22% of the global population, and its clinical signs include oral mucosa damage, tooth wear, masticatory muscle pain, or headache.[12–14] Even though SB is not considered a movement disorder,[11] it is associated with oxidative stress, decreased quality of life and sleep alteration.[15–17]

The exploration of specific sleep disorders and sleep structure in patients with migraine is a subject of research interest. The literature presents contrasting results concerning SB in migraine patients. Several studies have confirmed the relationship between SB and migraine,[18,19] but, on the other hand, there is a lack of association between SB diagnosis and migraine in studies based on video-polysomnography (vPSG), which is the gold standard for SB diagnosis.[15,20–22] Additionally, there is an absence of data regarding the association between SB and CM, as measured using objective methods. Only 1 study has demonstrated a close association between SB and CM.[19] However, it should be noted that SB was diagnosed in this study using questionnaires.[19] The association between SB and migraine is poorly explained, despite the increased prevalence of SB in individuals with migraine.[19,22] Moreover, patients with migraine often report reduced sleep quality. However, objective methods used to measure sleep architecture do not corroborate these complaints.[23,24] Yet, these results were calculated in comparison to patients without headaches; therefore, there is a paucity of studies assessing sleep structure in particular forms of migraine such as CM using vPSG.

In order to fill the gap concerning associations between SB, sleep structure and migraine forms, the main objective of the present study was to evaluate the associations between CM and SB using objective methods such as PSG and validated criteria for headache diagnosis, namely the 3rd edition of the International Classification of Headache Disorders (ICHD-3). The second aim was to assess sleep structure in CM patients in comparison to EM participants. We theorized that patients with CM may exhibit alterations in their sleep structure and diminished sleep efficacy. Additionally, we hypothesized that CM participants may have a greater number of SB episodes in comparison to patients with EM, and that SB is positively associated with CM.

Material and methods

The present study was approved by the Bioethics Committee of Wroclaw Medical University, Poland (approval No. KB/25/2024). The study was performed according to the principles outlined in the Declaration of Helsinki for experiments involving human participants.

Study participants

The participants of the study were patients at the Headache Center in the Department of Neurology at Wroclaw Medical University, Poland. The diagnosis of migraine was made based on the ICHD-3 criteria.[5] In order to exclude other potential causes of secondary headache, all patients underwent brain magnetic resonance imaging (MRI). Patients with CM received preventive treatment comprising topiramate, onabotulinumtoxinA and monoclonal antibodies (mAbs) targeting the calcitonin gene-related peptide (CGRP) pathway. None of the participants were undergoing active SB treatment in the form of pharmacotherapy, dental appliances or botulinum injections. The treatment of EM encompassed acute treatment in the form of non-steroidal anti-inflammatory drugs (NSAIDs) and triptans. The prophylactic treatment regimen was based on venlafaxine, amitriptyline, propranolol, and antihypertensive drugs. The aforementioned pharmaceuticals were administered in minimal doses when compared to standard therapeutic doses.[25] Interviews were conducted with the patients from the Headache Center,

with a focus on their sleep-related concerns. Reports of any sleep complaints allowed the referral of patients to the Sleep Laboratory at the Department of Diabetology, Hypertension, and Internal Diseases at Wroclaw Medical University for the diagnosis of sleep disorders.

Sleep architecture and sleep bruxism diagnosis

In the Sleep Laboratory, a single-night vPSG was performed on patients with suspected sleep disturbances. Sleep bruxism was diagnosed using vPSG, which is the gold standard for SB diagnosis.[26] Based on electromyography (EMG) recordings as well as audio and video recordings of the bilateral masseter, the following parameters were analyzed: the bruxism episode index (BEI); phasic bruxism, characterized by more than 2 cyclic phasic EMG increases lasting 0.25–2.00 s; tonic bruxism, characterized by episodes lasting >2 s; and mixed bruxism, which is a combination of the aforementioned types. The scoring of all SB episodes occurred under specific criteria, namely, when the activity exceeded at least twice the background EMG amplitude and remained above 3 s of stable EMG. Sleep bruxism was classified as mild (BEI: 2–4), severe (BEI > 4) or absent (BEI < 2). The vPSG device utilized was Nox-A1 (NOX Medical, Reykjavík, Iceland), which consisted of electroencephalography (EEG), electrooculography (EOG) and electrocardiography recordings. The pulse and oxygen saturation levels of the subjects were assessed using a pulse oximeter (WristOx$_2$® 3150; Nonin Medical Inc., Plymouth, USA). The American Academy of Sleep Medicine (AASM) standard criteria for sleep scoring were used by an experienced physician to analyze the vPSG recordings in 30-s epochs.[27] The evaluated sleep parameters were as follows: total sleep time (TST); sleep efficiency (SE); sleep latency (SL); wake after sleep onset (WASO); sleep stages, including non-rapid eye movement (NREM) (N1, N2, N3) and rapid eye movement (REM); percentage of TST spent in N1, N2, N3, and REM stages of sleep; apnea–hypopnea index (AHI); and periodic limb movement in sleep (PLMS).

Questionnaires

The participants completed questionnaires that measured their headache-related disability, anxiety, level of daytime sleepiness, level of stress, and habits (drinking coffee, tea or alcohol). Headache-related disability was measured using the Migraine Disability Assessment (MIDAS) questionnaire. The general level of daytime sleepiness was assessed with the Epworth Sleepiness Scale (ESS). The severity of anxiety was measured using the Generalized Anxiety Disorder Assessment (GAD-7). The Perceived Stress Scale (PSS) was used to evaluate the extent of stress experienced by the participants in their daily lives. Current alcohol consumption was confirmed if individuals diagnosed with migraine consumed any type of alcohol more than once per day. Current coffee and tea consumption was documented in patients with migraine who reported having at least 1 cup of tea or coffee during a 24-h period.

Eligibility criteria

The inclusion criteria for the present study were adult participants with a definitive migraine diagnosis and with technically appropriate single-night vPSG results who signed informed consent to participate in the study. The exclusion criteria were as follows: age <18 years; coexisting primary or secondary headaches; significant psychiatric, autoimmune and systemic diseases; migraine attack during the vPSG examination; lack of informed consent; absence of completed questionnaires describing stress, anxiety and headache-related disability; malignancies; pregnancy and lactation.

Statistical analysis

The STATISTICA software, v. 13.3 (TIBCO Software Inc., Palo Alto, USA), was used for the calculations. The compliance of the empirical distributions of continuous variables with the theoretical normal distribution was verified using the Shapiro–Wilk test. The homogeneity of variance of the results was checked using the Bartlett's test. For quantitative variables, medians (*Me*), lower quartiles (*Q1*) and upper quartiles (*Q3*) were calculated. The significance of differences in mean values (medians) of continuous variables with a non-normal distribution or with non-homogeneous variances in 2 independent groups was verified using the Mann–Whitney *U* test. Nominal and ordinal qualitative variables were presented in tables as numbers (*n*) and percentages (%). For categorical data, the Pearson's χ^2 test of independence or Fisher's exact test were used. Spearman's rank correlation coefficients (*rho*) or point biserial correlation (*rpb*) were used to assess the direction and significance of the relationship between 2 variables. To assess the influence of medications that interfere with sleep such as amitriptyline, venlafaxine and topiramate, used in the treatment of patients with migraine, a non-parametric analysis of covariance using the Quade test was performed. This analysis was conducted with the use of the R software with the npsm package (R Foundation for Statistical Computing, Vienna, Austria). For binary variables, Mantel–Haenszel adjusted odds ratios (*OR*s) were estimated to remove the influence of the confounding variable ("taking medications interfering with sleep"). All statistical hypotheses were verified using two-sided tests at a significance level of $p < 0.05$. Bonferroni correction was implemented in the data analysis. Prior to the study, no statistical power calculation was conducted.

Results

The study included 110 Caucasian patients with migraine: 97 (88%) females and 13 males, with a mean age of 39.3 years. There were 65 CM and 45 EM patients. The mean age of EM patients was 39.5 years and of CM patients – 39.1 years. The study population included 46 individuals with migraine with aura (MwA) and 64 migraine without aura (MwoA) patients. The patients with CM did not differ in terms of age, sex, body mass index (BMI), anxiety, stress, daytime sleepiness, current alcohol, tea or coffee consumption, medication intake such as amitriptyline, venlafaxine, topiramate, propranolol, or mAbs, and MwA occurrence in comparison to patients with EM ($p > 0.05$). The headache-related disability level, as measured by the MIDAS score, was increased in patients with CM (*Me*: 60.0 points vs. 24.0 points (EM patients), $p < 0.001$). The characteristics of the study sample are collated in Table 1.

Table 1. Characteristics of the study sample

Variable	EM patients ($n = 45$)	CM patients ($n = 65$)	*p*-value
Age [years] *M* ±*SD*	39.5 ±9.9	39.1 ±10.6	0.840
Female, *n* (%)	38 (84.4)	59 (90.8)	0.370
BMI [kg/m^2] *Me* (*Q*1; *Q*3)	23.8 (20.7; 25.7)	22.7 (20.5; 26.6)	0.940
Migraine with aura, *n* (%)	16 (35.6)	30 (46.2)	0.303
GAD-7 *Me* (*Q*1; *Q*3)	5 (3; 10)	6 (4; 9)	0.630
PSS *Me* (*Q*1; *Q*3)	22 (21; 25)	22 (21; 24)	0.960
ESS *Me* (*Q*1; *Q*3)	11 (6; 14)	8 (6; 13)	0.560
MIDAS *Me* (*Q*1; *Q*3)	24 (17; 34)	60 (44; 100)	<0.001*
Current alcohol consumption, *n* (%)	9 (20.0)	10 (15.4)	0.610
Current coffee consumption, *n* (%)	34 (75.6)	52 (80.0)	0.640
Current tea consumption, *n* (%)	18 (40.0)	37 (56.9)	0.120
Amitriptyline treatment, *n* (%)	5 (11.1)	5 (7.7)	1.000
Venlafaxine treatment, *n* (%)	9 (20.0)	5 (7.7)	0.060
Topiramate treatment, *n* (%)	3 (6.7)	4 (6.2)	1.000
mAbs treatment, *n* (%)	3 (6.7)	10 (15.4)	0.230
Propranolol treatment, *n* (%)	2 (4.4)	2 (3.1)	1.000
Taking medications that interfere with sleep architecture, *n* (%)	17 (37.8)	14 (21.5)	0.090

* statistically significant ($p < 0.05$, Mann–Whitney *U* test); EM – episodic migraine; CM – chronic migraine; *M* – mean; *SD* – standard deviation; BMI – body mass index; *Me* – median; *Q*1 – lower quartile; *Q*3 – upper quartile; GAD-7 – Generalized Anxiety Disorder Assessment; PSS – Perceived Stress Scale; ESS – Epworth Sleepiness Scale; MIDAS – Migraine Disability Assessment Scale; mAbs – monoclonal antibodies.

In terms of sleep structure among patients with migraine, a decline in the REM stage of sleep was observed in CM patients compared to individuals with EM (*Me*: 21.4% of TST vs. 24.4% of TST, respectively; $p = 0.008$). Other sleep parameters did not differ between the groups ($p > 0.05$) (Table 2).

Seventy-six out of 110 (69%) study participants were diagnosed with SB. There were 38 patients with mild SB (BEI > 2) and 38 patients with severe SB (BEI > 4). However, the median BEI was not increased in the CM group in comparison to the EM group (*Me*: 2.8 n/h vs. 2.6 n/h, respectively; $p = 0.400$). Additionally, the phasic, tonic and mixed SB episodes, as well as the duration of all SB episodes did not differ between the groups ($p > 0.05$). The characteristics of SB are presented in Table 3.

Factors associated with an increased likelihood of CM included REM sleep ≤23.1% of TST and mixed bruxism episodes ≥0.4 n/h (*aOR*: 3.61 (95% confidence interval (*CI*): 1.60; 8.15), $p = 0.002$; a*OR*: 2.40 (95% *CI*: 1.06; 5.46), $p = 0.048$, respectively). Cut-off values for REM sleep and mixed SB episodes were determined based on the analysis of receiver operating characteristic (ROC) curves (Fig. 1). Sleep bruxism (BEI > 2) was not associated with CM in comparison to EM (*aOR*: 0.73 (95% *CI*: 0.32; 1.65), $p = 0.290$) (Table 4).

Table 5 presents a comparison of population characteristics and sleep architecture between migraine patients with severe SB and those with mild SB. Increased age and

Table 2. Sleep structure in patients with migraine

Variable	EM patients ($n = 45$)	CM patients ($n = 65$)	*p*-value	Adjusted *p*-value
SE [%]	87.7 (82.2; 91.5)	85.6 (79.4; 89.4)	0.360	0.190
WASO [min]	47.6 (27.5; 73.0)	54.5 (34.8; 78.0)	0.360	0.210
SL [min]	11.8 (9.0; 25.0)	12.8 (6.1; 21.8)	0.850	0.990
N1 [% of TST]	3.4 (2.2; 5.3)	3.6 (2.6; 5.7)	0.320	0.380
N2 [% of TST]	51.9 (44.9; 56.0)	52.3 (47.2; 57.3)	0.460	0.280
N3 [% of TST]	20.3 (16.5; 24.3)	22.0 (15.2; 27.1)	0.360	0.560
REM [% of TST]	24.4 (20.4; 27.9)	21.4 (17.1; 24.4)	0.007*	0.008*
AHI [n/h]	2.5 (1.8; 4.2)	2.3 (1.1; 4.2)	0.240	0.870
PLMS [n/h]	1.7 (0.0; 4.3)	0.0 (0.0; 4.0)	0.180	0.770

Data presented as *Me* (*Q*1; *Q*3). * statistically significant ($p < 0.05$, Mann–Whitney *U* test); SE – sleep efficacy; WASO – wake after sleep onset; SL – sleep latency; N1–N3 – sleep stages; REM – rapid eye movement; TST – total sleep time; AHI – apnea–hypopnea index; PLMS – periodic limb movement in sleep; n/h – episodes per hour. Adjusted *p*-value is related to the administration of medications that interfere with sleep.

Table 3. Characteristics of sleep bruxism (SB) in patients with migraine

Variable	EM patients (*n* = 45)	CM patients (*n* = 65)	*p*-value	Adjusted *p*-value
BEI [n/h]	2.6 (2.0; 4.8)	2.8 (1.7; 4.8)	0.910	0.400
Phasic bruxism [n/h]	1.2 (0.6; 2.1)	1.1 (0.4; 2.1)	0.830	0.420
Tonic bruxism [n/h]	1.2 (0.8; 1.8)	1.2 (0.7; 1.8)	0.890	0.940
Mixed bruxism [n/h]	0.2 (0.0; 0.4)	0.3 (0.1; 0.7)	0.050	0.080
BEI average [s]	5.6 (4.6; 7.1)	6.0 (4.8; 7.6)	0.390	0.440
BEI maximum [s]	16.1 (11.0; 23.4)	15.9 (10.7; 23.0)	0.980	0.890
BEI minimum [s]	2.1 (2.0; 2.2)	2.0 (2.0; 2.2)	0.760	0.730
Phasic average [s]	6.2 (4.6; 8.4)	6.1 (5.0; 8.3)	0.880	0.870
Phasic maximum [s]	13.5 (7.5; 18.6)	12.0 (7.0; 18.8)	0.660	0.690
Phasic minimum [s]	2.8 (2.3; 3.4)	2.7 (2.0; 3.8)	0.610	0.350
Tonic average [s]	4.2 (3.4; 4.8)	3.9 (3.3; 4.9)	0.700	0.790
Tonic maximum [s]	8.4 (5.6; 11.1)	8.8 (6.3; 10.9)	0.810	0.770
Tonic minimum [s]	2.1 (2.0; 2.2)	2.1 (2.0; 2.3)	0.340	0.230
Mixed average [s]	8.7 (0.0; 11.8)	10.2 (6.1; 12.7)	0.280	0.400
Mixed maximum [s]	10.6 (0.0; 16.3)	12.5 (7.0; 18.1)	0.300	0.350
Mixed minimum [s]	5.3 (0.0; 8.4)	6.4 (3.0; 8.8)	0.280	0.680

Data presented as *Me* (*Q*1; *Q*3). BEI – bruxism episode index. Adjusted *p*-value is related to the administration of medications that interfere with sleep.

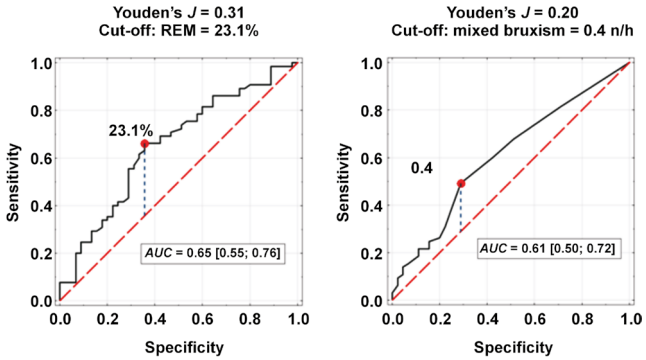

Fig. 1. Receiver operating characteristic (ROC) curves for predicting the occurrence of chronic migraine (CM) based on the duration of rapid eye movement (REM) sleep and the presence of mixed bruxism episodes

AUC – area under the ROC curve.

Table 4. Factors associated with chronic migraine (CM)

Factor	EM patients (*n* = 45)	CM patients (*n* = 65)	*p*-value	*OR* (95% *CI*)	*aOR* (95% *CI*)
REM ≤23.1%	43 (66.2%)	16 (35.6%)	0.002*	3.54 (1.60; 7.87)	3.61 (1.60; 8.15)
Mixed bruxism ≥0.4 n/h	32 (49.2%)	13 (28.9%)	0.048*	2.39 (1.06; 5.35)	2.40 (1.06; 5.46)
SB (BEI > 2)	42 (64.6%)	34 (75.6%)	0.290	0.59 (0.25; 1.38)	0.73 (0.32; 1.65)

* statistically significant ($p < 0.05$, Fisher's exact test); *CI* – confidence interval; *OR* – odds ratio; *aOR* – adjusted *OR* related to the administration of medications that interfere with sleep.

Table 5. Characteristics of patients with migraine in groups with mild and severe sleep bruxism (SB)

Variable	Patients with migraine		*p*-value	Adjusted *p*-value
	mild SB (BEI: 2–4) (*n* = 38)	severe SB (BEI > 4) (*n* = 38)		
Age [years] *M* ±*SD*	40.5 ±10.4	35.5 ±10.9	0.040*	0.049*
Female, *n* (%)	36 (94.7)	30 (78.9)	0.090*	0.048*
GAD-7 *Me* (*Q*1; *Q*3)	6 (2; 11)	7 (4; 10)	0.350	0.640
PSS *Me* (*Q*1; *Q*3)	22 (21; 25)	22 (21; 24)	0.990	0.800
ESS *Me* (*Q*1; *Q*3)	8.5 (5; 14)	9 (7; 15)	0.430	0.390
Migraine with aura, *n* (%)	12 (31.6)	21 (55.3)	0.040*	0.044*
CM, *n* (%)	18 (47.4)	24 (63.2)	0.250	0.170
MIDAS *Me* (*Q*1; *Q*3)	34.0 (23; 70)	47.5 (27; 72)	0.340	0.980
SE [%] *Me* (*Q*1; *Q*3)	86.8 (80; 92)	85.6 (78; 92)	0.900	0.780
WASO [min] *Me* (*Q*1; *Q*3)	54.8 (33; 79)	50.8 (24; 76)	0.590	0.670
SL [min] *Me* (*Q*1; *Q*3)	11.6 (7; 20)	12.3 (9; 26)	0.590	0.410
N1 [% of TST] *Me* (*Q*1; *Q*3)	3.5 (2.3; 5.3)	3.5 (2.5; 7.5)	0.280	0.180
N2 [% of TST] *Me* (*Q*1; *Q*3)	50.9 (43; 56)	52.7 (48; 60)	0.220	0.150
N3 [% of TST] *Me* (*Q*1; *Q*3)	21.0 (16; 27)	21.6 (15; 24)	0.520	0.230
REM [% of TST] *Me* (*Q*1; *Q*3)	24.2 (18; 27)	22.2 (18; 25)	0.130	0.130
AHI [n/h] *Me* (*Q*1; *Q*3)	2.3 (1.6; 3.9)	2.3 (1.1; 5.1)	0.740	0.160
PLMS [n/h] *Me* (*Q*1; *Q*3)	0.5 (0.0; 4.3)	2.2 (0.0; 4.4)	0.330	0.610

* statistically significant ($p < 0.05$, Mann–Whitney *U* test and Fisher's exact test). Adjusted *p*-value is related to the administration of medications that interfere with sleep.

female sex were more frequent in migraine patients with mild SB in comparison to severe SB (mean (*M*): 40.5 years vs. 35.5 years, adjusted $p = 0.049$; 94.7% of females vs. 78.9% of females, adjusted $p = 0.048$, respectively). As illustrated in Table 6, severe SB (BEI > 4) was associated with MwA in comparison to MwoA (*OR*: 2.68 (95% *CI*: 1.05; 6.83); adjusted $p = 0.044$).

Table 7 presents the values for Spearman's rank correlation coefficient (*rho*) and point biserial correlation coefficient (*rpb*) between BEI (n/h) and female sex, CM, MwoA, MIDAS score, and age. Migraine without aura, as well as female sex and age demonstrated a weak negative correlation with BEI ($r = -0.293$, $p = 0.002$; $r = -0.246$, $p = 0.010$; and $r = -0.268$, $p = 0.005$, respectively). The MIDAS score and CM diagnosis were not correlated with BEI ($r = 0.071$, $p = 0.458$; and $r = -0.012$, $p = 0.904$, respectively).

Table 6. Association between severe sleep bruxism (SB) and migraine with aura (MwA)

Bruxism severity	MwA (*n* = 33)	MwoA (*n* = 43)	Adjusted *p*-value	*OR* (95% *CI*)
Severe SB (BEI > 4)	21 (63.6%)	17 (39.5%)	0.044*	2.68 (1.05; 6.83)
Mild SB (BEI: 2–4)	12 (36.4%)	26 (60.5%)		1.00 (ref.)

* statistically significant ($p < 0.05$, Fisher's exact test); MwoA – migraine without aura.

Table 7. Association between bruxism episode index (BEI) and chosen parameters using Spearman's rank correlation coefficient (*rho*) and point biserial correlation coefficient (*rpb*)

Parameter	*rho* or *rpb* (95% *CI*)	*p*-value
Female sex	–0.246 (–0.265; –0.227)	0.010*
CM	–0.012 (–0.198; 0.176)	0.904
MwoA	–0.293 (–0.455; –0.112)	0.002*
MIDAS	0.071 (–0.117; 0.255)	0.458
Age	–0.268 (–0.434; –0.085)	0.005*

* statistically significant ($p < 0.05$).

Discussion

Sleep structure in chronic migraine

Sleep structure is altered in patients with CM in comparison to those with EM. A reduction in REM sleep was noted in these patients. Therefore, the null hypothesis was partially confirmed, because apart from REM sleep, no significant alterations were observed in other sleep parameters. It has been established that REM sleep is decreased in patients with migraine.[23] However, a recent meta-analysis suggests that other sleep parameters remain unaltered in individuals with migraine.[23] This is an interesting fact given that patients with migraine often report sleep disturbances, which are not confirmed by vPSG.[23,24] These results were made based on the comparison of migraine patients with healthy controls. However, there are rare reports comparing sleep structure based on migraine subtypes using vPSG. Research has demonstrated that CM reduces NREM3 duration and sleep efficiency in comparison to chronic tension-type headache (TTH).[28] Orzeszek et al. suggested that the severity of orofacial pain and headaches is not associated with alterations in sleep quality.[29] In the present study, the CM group demonstrated a decrease in REM sleep, the role of which is memory consolidation, dreaming, thermoregulation, and emotional processing.[30] Previous studies have proven that migraine is associated with dysfunction of memory processing.[31] Therefore, it is possible that patients with migraine have impaired memory processing resulting from the decreased amount of REM sleep. However, it must be reiterated that these results are calculated in comparison to the control group, which does not include individuals with migraine. The findings of this study indicate that CM patients may exhibit impaired memory processing compared to patients with EM due to reduced duration of REM sleep. However, there is a paucity of literature focusing on this topic, and further research must be conducted to explain this theory. At the same time, the diagnosis of SB is associated with alterations in sleep structure.[32] Severe SB (BEI > 4) has been shown to decrease NREM3 sleep while increasing NREM1 and REM sleep.[33] However, in our study, there were no differences in sleep structure following the division of patients with migraine based on SB severity. Thus, it appears that SB does not alter sleep in patients with migraine. Migraine treatment may also have an influence on sleep structure, because it consists of the administration of amitriptyline, venlafaxine and topiramate, which can affect sleep.[34,35] However, this confounding factor was considered in the analysis and, after adjustment, the results still showed that REM sleep was decreased in patients with CM. Therefore, the observed reduction in REM sleep may be unique to migraine chronification.

Sleep bruxism in migraine

A high prevalence of SB was observed in migraine, with only mixed SB episodes above 0.4 n/h demonstrating a correlation with CM. Similar results were observed in a study that explored the associations between SB and migraine in a temporomandibular disorder (TMD) group using vPSG.[22] Particular types of SB episodes are still subjects of research, but it has been shown that tonic SB episodes are associated with sleep-related breathing disorders.[36] Therefore, it is necessary to explore this topic in further studies and establish whether mixed episodes are one of the hallmarks of migraine or are concomitant findings among patients with migraine. Despite the fact

that the present study was focused on the associations between SB and CM, it was discovered that severe SB was related to MwA. Additionally, a weak and negative correlation was identified between BEI and MwoA. Aura is described as a transient neurological symptom that occurs from 5 to 60 min prior to the onset of a headache attack. The most common form of aura are visual disturbances.[37] The pathopsychological theory of aura embraces cortical spreading depression and changes in brain vasculature.[38] Previous research has indicated that SB may be centrally regulated from the brainstem.[39] Contemporary understanding suggests that the etiology of SB is multifactorial.[40] These results imply the potential of shared etiology between aura and SB. However, further studies are needed in this regard.

Association between chronic migraine and sleep bruxism

Contrary to the null hypothesis of this study, no association was found between overall SB and CM. However, previous research has demonstrated a positive correlation between CM and SB, with the co-occurrence of TMD in SB further increasing this association.[19] The diagnosis of SB in the mentioned study was based on questionnaires, and these associations were calculated in comparison to a control group without headaches.[19] This difference may also stem from the fact that, in our study, SB was diagnosed using vPSG, and our primary objective was not focused on identifying and including TMD conditions. Additionally, no correlation was observed between BEI values, which are used in SB diagnosis, and MIDAS scores. However, MIDAS scores did not differ between patients presenting with migraine and severe SB and those with mild SB. Generally, patients with CM have significantly increased life disability due to headaches, with this severity being associated with the MIDAS score.[41] Therefore, the lack of association between BEI and MIDAS may indicate that the overall SB diagnosis is not associated with migraine. However, Memmedova et al. demonstrated that patients with bruxism and combined headaches, including migraine, TTH and trigeminal autonomic cephalalgia (TAC) exhibited increased MIDAS scores in comparison to those without bruxism.[42] However, the mentioned study was not based on vPSG, and the study group included more than one type of headache. Therefore, taking into consideration all the presented aspects, SB may be related to aura phenomena in migraine rather than migraine chronification.

Limitations

Despite the use of vPSG and the ICHD-3 criteria in the present study, some limitations must be considered. First, the sample size was constrained due to the limited accessibility of vPSG. Prior to the study, a statistical power calculation was not performed. Additionally, the questionnaires may not adequately capture the level of stress, headache-related disability and anxiety in patients with migraine. The temporal relationships between SB and CM were not examined. Patients with migraine received treatment in various forms, and these may have influenced the observed results. In SB like in migraine, botulinum is considered an effective treatment; however, doses of other toxins and other regions of the face muscles are included in the treatment of SB.[43] Additionally, the first-line SB therapy is pharmacotherapy, dental appliances or behavioral methods rather than botulinum injections[44]; therefore, the influence of botulinum on SB results may be non-relevant. Video-polysomnography was conducted without an adaptive night, and the influence of the first-night effect on vPSG results may be significant. Therefore, the presented observations must be interpreted with caution.

Conclusions

A decrease in REM sleep was associated with CM. Despite the high prevalence of SB in patients with migraine, SB was not associated with CM, while severe SB was associated with MwA. Therefore, if any association between SB and migraine exists, it is more related to aura phenomena than to migraine chronification. Further studies are necessary to elucidate these results and to better understand the relationship between sleep disturbances in CM.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee at Wroclaw Medical University, Poland (approval No. KB/25/2024).

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

Not applicable.

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Original paper

Effects of endodontic retreatment by conventional therapy compared to combined therapy with an Er:YAG laser and photobiomodulation: A randomized clinical trial

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Abstract

Background. The success of endodontic retreatment relies on the effective elimination of pathogenic microflora from the root canal.

Objectives. The study aimed to investigate the effects of an erbium-doped yttrium-aluminum-garnet (Er:YAG) laser and a 635-nm laser on the healing of asymptomatic chronic periapical lesions (PLs) in endodontically treated teeth and the reduction of postoperative pain.

Material and methods. Forty patients with PLs in mandibular molars were referred for root canal retreatment (RCR). Conventional chemo-mechanical endodontic treatment was conducted in the control group (G1; $n = 20$). In the test group (G2; $n = 20$), in addition to conventional chemo-mechanical treatment, Er:YAG laser-activated irrigation (LAI) with 2% NaOCl and 17% EDTA was performed. The laser parameters were as follows: 50 mJ; 25 Hz; 1 W; 300 µs; a tip diameter of 300 µm; fluence of 71.4 J/cm^2; and power density of 1,428.6 W/cm^2. Subsequently, the canals were filled with thermo-condensed gutta-percha, using the AH Plus sealer. In group G2, additional photobiomodulation (PBM) with a wavelength of 635 nm (400 mW, 5 s per point, a dose per point: 2 J, a dose per square centimeter: 4 J, an applicator diameter of 8 mm) was applied, with 2 application points at the apex level, administered over 4 sessions – on the treatment day, and after 24 h, 48 h and 96 h. Endodontic lesion remission was assessed by measuring the PL size with the use of cone-beam computed tomography (CBCT) at 6 and 12 months postoperatively. Postoperative pain was evaluated using the visual analog scale (VAS) after 1, 2 and 4 days.

Results. The study results demonstrated a statistically significant decrease in the mean PL size at 6 months postoperatively in the test group (mean PL size: 1.55 ±0.51 mm) as compared to the control group (mean PL size: 1.95 ±0.71 mm) ($p < 0.05$). In the test group, postoperative pain on VAS was significantly lower after the procedure ($p < 0.05$).

Conclusions. The application of Er:YAG and 635-nm diode lasers improved PL healing and decreased postoperative pain.

Keywords: periapical periodontitis, photobiomodulation, endodontics, erbium laser, Er-YAG

Highlights

- Enhanced lesion healing: The application of Er:YAG laser-activated irrigation (LAI) significantly accelerated the healing of periapical lesions (PLs) at 6 months as compared to conventional endodontic retreatment.
- Pain reduction: Photobiomodulation (PBM) with a 635-nm diode laser significantly reduced postoperative pain at 24 and 48 h post-treatment.
- Synergistic laser effect: Combining Er:YAG LAI with 635-nm PBM provided superior clinical outcomes in both infection control and patient comfort.
- Effective disinfection: Laser-activated irrigation resulted in deeper irrigant penetration and better debridement of root canal complexities, supporting more effective microbial elimination.
- Clinical benefit: This combined laser approach offers an evidence-based adjunct to traditional endodontic retreatment, improving both the healing rates and patient experience.

Introduction

The failure rate in endodontic therapy typically ranges from 2% to 16%.[1] The success of root canal therapy relies on the effective elimination of pathogenic microflora from the root canal.[2,3] In comparison with primary treatment, the revision of endodontic procedures poses greater challenges due to technical hurdles in canal preparation and the persistent activity of intracanal microorganisms.[4] Bacterial infection not only results in chronic apical periodontitis (CAP), but also heightens the potential for postoperative discomfort. The fundamental objective of endodontic treatment is to thoroughly eliminate the pathogenic microorganisms residing within the intricate network of the root canal system, thereby promoting the restoration of optimal oral health and preventing potential reinfection. However, the achievement of effective disinfection can be challenging due to the intricate nature of the root canal system, which encompasses features like isthmuses, oval extensions and lateral canals.[1,2,4]

Teeth affected by apical periodontitis (AP) pose a greater challenge in terms of endodontic treatment as compared to those without lesions during the treatment phase.[5] This complexity primarily arises from the persistent bacterial activity within the root canal system. The time required for a lesion to heal varies between 6 months and 2 years, occasionally extending even further.[6] Sathorn and Parashos stated that if a lesion remains unresolved for 4 years, spontaneous resolution becomes unlikely.[6] Indeed, if a lesion maintains its size or expands after a year of follow-up, or if a lesion appears in a tooth that previously exhibited none, root canal retreatment (RCR) is warranted.[7] Radiographically, the complete healing of the cortical bone and a healthy appearance within 6–24 months post-treatment signify the success of the intervention.[8] Nevertheless, cases of post-treatment AP can emerge even when the initial treatment was executed correctly, due to various factors, such as the presence of anatomical complexities, an incomplete removal of the infected tissue, undetected accessory canals, or microbial resistance, emphasizing the necessity for vigilant follow-up and potential retreatment to ensure successful outcomes. The occurrence of post-treatment affliction is discerned in 5–15% of cases previously exhibiting pre-treatment AP, regardless of adherence to proper procedural standards.[9] The smear layer, which permeates dentinal tubules up to 40 μm along with dentin debris, tends to deactivate root canal medicaments and irrigants while obstructing their penetration into the biofilm.[10] Thus, the imperative to eliminate the smear layer and debris cannot be overstated, as far as averting AP is concerned.

Clinicians employ diverse methodologies to ensure precise cleaning and disinfection in endodontic retreatment.[2,11–13] In this context, various irrigation systems have become integral for achieving improved treatment outcomes, addressing the challenges associated with the failure rates in endodontic retreatment cases.[14] Achieving comprehensive root canal decontamination is imperative, requiring the meticulous removal of gutta-percha and the sealer not only from the main canal, but also from anatomically intricate lateral canals or fins. This meticulous approach ensures thorough disinfection and prevents potential microbial reservoirs, contributing to the success of endodontic treatment.[15] While the existing studies have primarily focused on the removal of calcium hydroxide ($Ca(OH)_2$) and debris from the root canal and the simulated lateral canals,[16] there is still a notable gap in the literature concerning the specific removal of the sealer, using varied irrigation methodologies. Recent advancement has introduced various irrigation systems and techniques.[17–19] RinsEndo, characterized by a hydrodynamic mechanism involving alternating positive and negative pressure, induces macroscopic and microscopic blistering to activate the irrigant, thereby enhancing its efficacy.[19] Approaches such as passive ultrasonic irrigation and the EndoVac™ system leverage negative pressure to actively administer and remove irrigation solutions, ensuring thorough cleansing.[17,19] The CanalBrush endodontic instrument, a malleable plastic micro-brush integrated into a dental handpiece operating at 600 rpm, in conjunction with manual irrigation, presents a promising yet inconclusive

approach, with varying conclusions across the existing studies.[20] Conversely, EndoActivator, a battery-powered handpiece featuring a non-cutting polymer tip, achieves the sonic activation of the intracanal irrigant.[20] Widely adopting an oscillating, non-cutting ultrasonic tip for activation, the available literature consistently validates the heightened efficacy of ultrasonically activated irrigation (UAI).[19] Both ultrasonic and sonic irrigation methods capitalize on acoustic vibrations to dislodge debris, thereby bolstering the efficacy of irrigation solutions.[19,21] However, a study by van der Sluis et al. indicates the potential for file breakage and the limited penetration in curved canals.[18] In turn, laser activation employs laser energy for irrigant activation.[21]

A distinctive method for activating irrigants in endodontic procedures is laser-activated irrigation (LAI), leveraging pulsed erbium, chromium-doped yttrium-scandium-gallium-garnet (Er,Cr:YSGG) and erbium-doped yttrium-aluminum-garnet (Er:YAG) lasers. These lasers induce optical cavitation within the irrigant, causing the formation of expanding and imploding vapor bubbles at the fiber tip, with smaller secondary bubbles emerging deeper in the canal undergoing acoustic streaming.[22,23] Notably, controlled laboratory studies have reported an impressive 99.5–100% reduction in bacterial load within the infected root canals through the application of LAI.[24,25] Furthermore, rigorous laboratory investigations have demonstrated the heightened efficacy of LAI with the Er:YAG laser over conventional irrigation and UAI, specifically in removing debris from intricate canal structures.[23,26] However, it is essential to note that the outcomes of studies do not uniformly reveal a statistically significant difference between LAI and UAI. Furthermore, photobiomodulation (PBM) emerges as a promising intervention in mitigating postoperative pain following root canal treatment.[27,28] By harnessing the properties of the laser wavelength within the optical window, PBM has demonstrated efficacy in reducing postoperative pain associated with root canal treatment.[29] The application of low-level laser therapy (LLLT) adheres to 3 fundamental principles: biostimulation; analgesia; and the modulation of inflammatory processes.[30–37] This therapeutic modality has demonstrated efficacy in mitigating postoperative pain and edema following the extraction of impacted mandibular third molar teeth,[38] managing dentine hypersensitivity,[39] alleviating postoperative pain after endodontic surgery,[40] addressing postoperative pain subsequent to RCR,[27,41] and attenuating postoperative pain following the initial root canal treatment of mandibular molar teeth with symptomatic CAP.[42]

The principal aim of the present investigation was to examine the synergistic effect resulting from the application of an Er:YAG laser in conjunction with a 635-nm diode laser, specifically assessing their combined influence on the remission of periapical lesions (PLs). Additionally, the study aimed to evaluate the potential mitigation of postoperative pain associated with this combined laser approach. The null hypothesis in the present study was that there would be no differences in the PL size and the pain experienced by patients at 24 h, 48 h and 96 h post-treatment after the application of Er:YAG LAI and PBM as compared to endodontic retreatment using classical methods.

Material and methods

The trial was structured as a randomized and controlled study. Approval was obtained from the Local Ethics Committee at the Regional Medical Chamber (permission No.: 318/KBL), and informed consent was acquired from all the participating subjects in line with the principles of the Declaration of Helsinki.

Subjects

All patients underwent treatment at a dental office, under the care of the same endodontist (I.K-B.). Patients' randomization was done using the https://www.randomizer.org webpage. The average age of the participants was 45.3 ±9.8 years. The sample size of 20 subjects in each group was determined using the G*Power software from Kiel University, Germany. The calculations were based on our preliminary tests, considering a significance level of 0.05, an effect size (d) of 0.81 and a power of 80%. The sample size was calculated based on our preliminary results with a smaller number of patients, and on a similar study related to this topic.[27]

The allocation of participants into 2 groups followed a 1:1 ratio and was carried out using a coin toss. The selection criteria for the cases were as follows: teeth treated endodontically, with PLs having a diameter of less than 10 mm; overall good health; a diagnosis of asymptomatic CAP in mandibular molars, with a favorable prognosis for functional reconstruction post-endodontic treatment; and primary endodontic treatment performed a minimum of 4 years prior to the current trial. The exclusion criteria encompassed systemic disorders, pregnancy and breastfeeding, periodontal diseases (pocket depth (PD) of over 3 mm), the use of analgesic agents within 5 days before the procedure, the administration of antibiotics up to 1 month before the procedure, teeth affected by post-trauma complications and those with the materials extending beyond the apical foramen, teeth with broken instruments, teeth with root resorption, and type 3 of canal curvature according to Schneider's classification. To maintain the integrity of the study, blinding was implemented during the recording of postoperative pain evaluations and cone-beam computed tomography (CBCT) assessments. This ensured that the individuals responsible for gathering and assessing this data remained unaware of treatment conditions or group assignments, minimizing bias and enhancing the reliability of the study outcomes.

Clinical procedure

A total of 40 patients requiring root endodontic retreatment in mandibular molars were randomly divided into 2 groups using a coin toss, based on the treatment procedure. The control group (G1; n = 20) received conventional chemo-mechanical endodontic treatment. In the test group (G2; n = 20), conventional treatment was supplemented with the application of 2 lasers: an Er:YAG laser (AdvErL EVO; J. Morita, Ina-machi, Japan) for activating the irrigant; and a 635-nm diode laser (SMARTmPRO; Lasotronix, Piaseczno, Poland) for postoperative PBM. In the control group, EndoActivator (EndoActivator System Kit; Dentsply Sirona, Bensheim, Germany) was employed for the activation of the irrigant (Fig. 1).

After administering anesthesia, using 1.8 mL of 4% articaine (Citocartin 200 with 1:200,000 epinephrine; Molteni Dental, Milan, Italy), and removing the filling material with a diamond bur on a high-speed, contra-angle handpiece, a rubber dam was placed. Before commencing the cleaning and shaping procedures for endodontic treatment, each tooth crown was reconstructed with a composite material. After preparing access to the tooth chamber, the tooth cavity was irrigated with 2% sodium hypochlorite (NaOCl). The activation of the irrigant was performed using either EndoActivator (30 s, 15/02 tip) or the Er:YAG laser (20 s, R300T tip) by placing the tips into the tooth chamber following each instrumentation with the file.

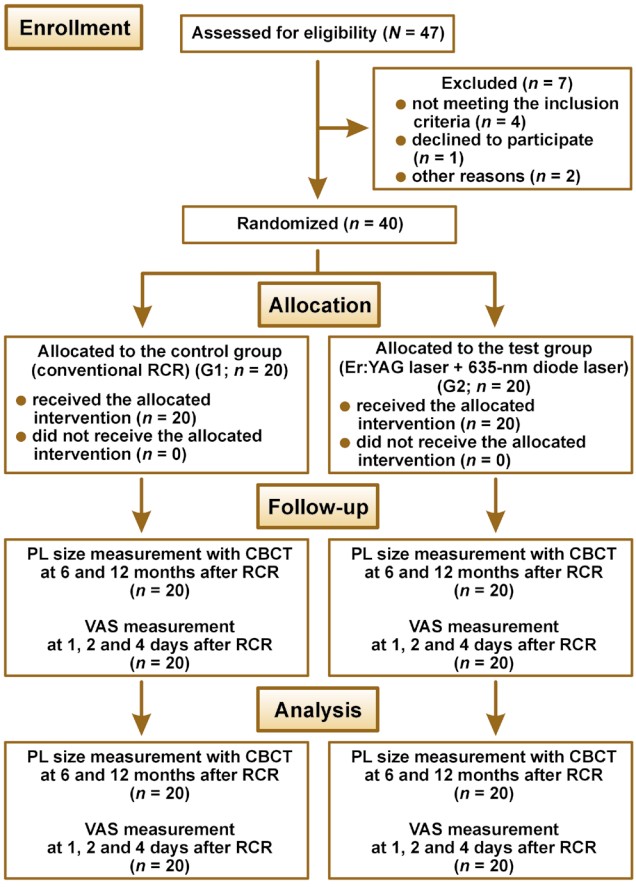

Fig. 1. Flow chart of the treated subjects according to the CONSORT (Consolidated Standards of Reporting Trials) 2010 statement

RCR – root canal retreatment; Er:YAG laser – erbium-doped yttrium-aluminum-garnet laser; PL – periapical lesion; CBCT – cone-beam computed tomography; VAS – visual analog scale.

The dissolution of the endodontic filling material (gutta-percha) was accomplished through the application of orange oil. Subsequent to achieving canal negotiation by utilizing C-pilot files (VDW, Munich, Germany), and confirming the attainment of the full working length through measurements with the Raypex 6 apex locator (VDW) and radiographic verification featuring an endodontic instrument positioned within the root canal, the canals underwent irrigation with 2% NaOCl. The Er:YAG laser R300T tip was positioned at a depth of 3–5 mm from each tooth canal orifice. In contrast, EndoActivator was placed into the root canal as deeply as possible after canal instrumentation, not exceeding 2 mm before the working length.

Hand instruments were utilized until achieving a canal size of 20/02, after which rotary instrumentation (E3 Azure Endostar files; Poldent, Warsaw, Poland; X-Smart® Plus; Dentsply Sirona), operating at a speed of 300 rpm, with a torque set between 2.1 and 3.0 N·m, was employed until attaining a size of 35/04.

After completing the remaining canal preparation up to a size of 35/04, rinsing was conducted using 10 mL of NaOCl and 2 mL of 17% ethylenediaminetetraacetic acid (EDTA) on each canal, using either EndoActivator or the Er:YAG laser. Final irrigation using an endodontic needle (positioned 2 mm short of the working length) was performed with 2 mL of a saline solution per canal, without activation. After achieving dryness, the canals were then obturated with thermally condensed gutta-percha and the AH Plus sealer (Dentsply Sirona). The teeth were temporarily restored using a composite material. In all cases, single-visit treatment was performed (Fig. 2).

Laser application

The 2,940 nm wavelength of the Er:YAG laser was utilized for LAI, with the following parameters: energy – 50 mJ; frequency – 25 Hz; power – 1 W; pulse width – 300 µs; tip diameter – 300 µm; fluence – 71.4 J/cm^2; and power density – 1,428.6 W/cm^2. The duration of LAI was 20 s.

The PBM of the periapical area was performed immediately after endodontic treatment, using the 635-nm diode laser with the following parameters: power – 400 mW; time – 5 s per point; dose per point – 2 J; dose per square centimeter – 4 J; applicator diameter – 8 mm; spot area – 0.5024 cm^2; average power density – 0.8 W/cm^2; 2 application points at the buccal and lingual apex levels; 4 sessions – immediately after RCR, and subsequently after 24 h, 48 h and 96 h.

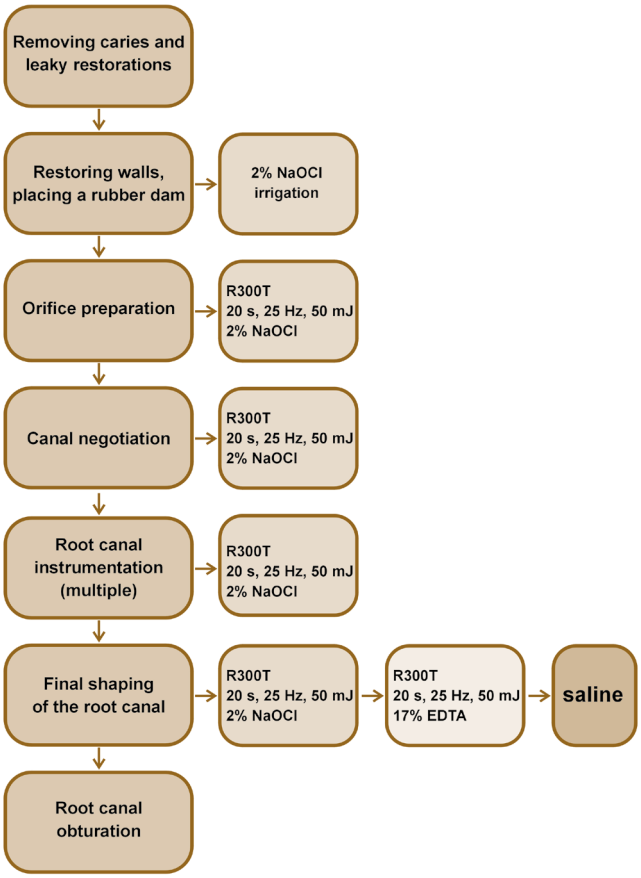

Fig. 2. Irrigant activation protocol using the Er:YAG laser during the root canal retreatment (RCR) procedure

EDTA – ethylenediaminetetraacetic acid.

Measurement of periapical lesions

Endodontic lesion remission was evaluated using CBCT (CS 8100 3D; Carestream Dental, Marne-la-Vallée, France). The PL size was measured in various dimensions, and the largest measured dimension of the lesion was considered for calculations. The measurements were taken at baseline (before treatment), as well as at 6 and 12 months postoperatively. The initial size range of lesions was similar for both groups – 3.7–5.9 mm and 3.4–6.0 mm for groups G2 and G1, respectively.

Measurement of postoperative pain

Postoperative pain assessment was conducted utilizing the visual analog scale (VAS) at specific intervals – 1, 2 and 4 days post-procedure. The VAS, a numerical scale ranging from 0 (denoting the absence of pain) to 10 (indicating unbearable pain), served as the instrument for quantifying the pain levels. The patients were administered structured questionnaires to articulate their pain experience on the designated days. The collated responses were gathered during the subsequent follow-up visit scheduled 7 days post-procedure, contributing to a comprehensive evaluation of postoperative pain dynamics (Fig. 3).

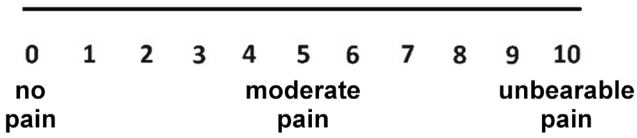

Fig. 3. Visual analog scale (VAS) for pain assessment

Statistical analysis

The normality of the data was assessed using the Kolmogorov–Smirnov test. Since the data distribution exhibited normality, statistical analysis for the PL size and the VAS pain level was conducted using Student's *t* test. This analysis was performed with IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA), with a significance level set at $p = 0.05$.

Results

Periapical lesion healing following root canal retreatment

The assessment of the PL size, measured in millimeters, after 6 months revealed a significant difference in the reduction of the lesion dimensions between the control subjects (mean PL size: 1.95 ±0.71 mm) and the test group (mean PL size: 1.55 ±0.51 mm) ($p < 0.05$). However, the results obtained after 1 year demonstrated insignificant improvement, i.e., a greater reduction in the PL size, following the application of lasers (mean PL size: 0.90 ±0.64 mm) in comparison with the control group (mean PL size: 1.11 ±0.66 mm) ($p > 0.05$) (Table 1, Fig. 4).

Pain level following root canal retreatment

The reduction in postoperative pain was assessed in both groups at 1, 2 and 4 days after the procedure.

Table 1. Mean differences in the periapical lesion (PL) size [mm] after 6 and 12 months in both groups

Time point	G1	G2	*t*	df	*p*-value
After 6 months	1.95 ±0.71	1.55 ±0.51	–2.02	37	0.025*
After 12 months	1.11 ±0.66	0.90 ±0.64	–0.99	37	0.165

Data presented as mean ± standard deviation (*M* ±*SD*).
Groups: G1 –control group; G2 – test group. df – degrees of freedom; * statistically significant (Student's *t* test for independent samples).

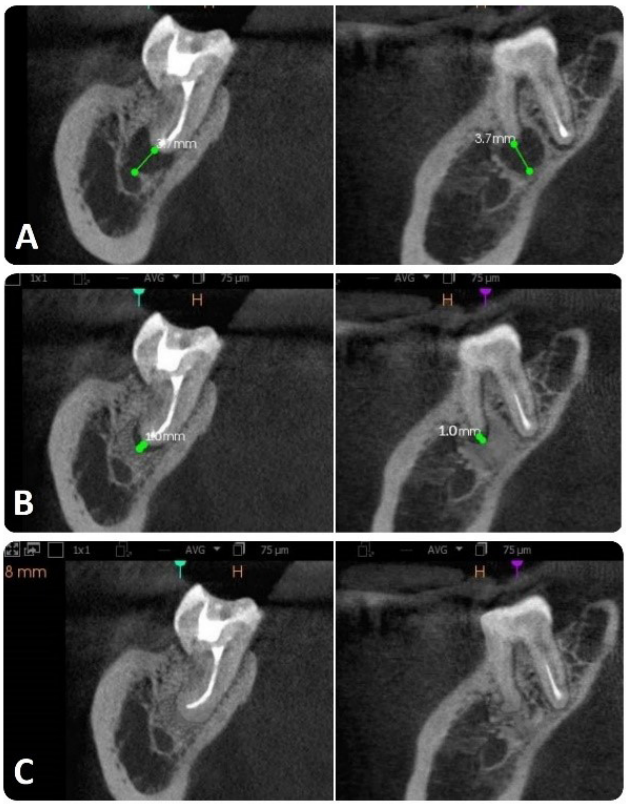

Fig. 4. Cone-beam computed tomography (CBCT) imaging of tooth 47 (FDI World Dental Federation classification)

A – before treatment; B – at the 6-month follow-up; C – at the 12-month follow-up.

The mean VAS scores for pain were significantly lower in group G2 (3.11 ±1.29 and 2.16 ±0.96) as compared to group G1 (4.10 ±1.86 and 2.95 ±1.39) at the 24-hour and 48-hour marks, respectively ($p < 0.05$). However, the VAS scores at 4 days (96 h) showed an insignificant difference between the groups ($p > 0.05$) (Table 2).

Discussion

The null hypothesis in the present study regarding the lack of differences in the PL size change after RCR when applying Er:YAG LAI and PBM was rejected. In terms of postoperative pain reduction, the null hypothesis about the lack of differences between the test and control groups was partially rejected. Our present findings showed a significant reduction in the average PL size at 6 months of Er:YAG laser- and PBM-assisted RCR as compared to conventional secondary root endodontic therapy. Furthermore, the pain reduction in the test group measured after 24 h and 48 h was significantly greater as compared to the control group, while the difference was insignificant at 96 h after treatment. The energy of the erbium laser induces cavitation, triggering an effervescent effect on the irrigant, thereby significantly enhancing its disinfection efficacy – the volume of the activated irrigant can surge up to 1,600 times.[21] Moreover, the application of non-ablative photonic energy through PBM modulates biological processes within tissues, affecting the larger biological system. This modulation extends to cellular metabolism, leading to secondary effects that alter cellular behavior.[43]

The primary aim of the study was to assess the effectiveness of Er:YAG LAI. The photoacoustic effect evoked in the fluids irradiated with the tip placed in a tooth chamber enables precise cleaning of the root canal and removes the smear layer produced by the mechanical shaping of canal dentin with endodontic instruments.[44] The research conducted by van der Sluis et al. revealed that LAI involved stimulating irrigants to heighten their penetration into dentinal tubules.[18] This stimulation renders the irrigant more reactive, enabling it to flow in a three-dimensional (3D) manner within root canals, thereby augmenting its antibacterial and cleansing effects.[18,23,45] In the present study, the application of LAI serves the precise and effective removal of old endodontic material from root canals. In other studies, LAI has demonstrated positive outcomes in bolstering the efficiency of the mechanical elimination of the root canal treatment filling and enhancing the disinfection attributes of irrigants.[23,45] In a study by Neelakantan et al., 3 different irrigation protocols utilizing distinct activation methods (ultrasound, and diode and erbium lasers) were evaluated on mature *Enterococcus faecalis* biofilms.[44] The study determined that energizing the irrigants with laser energy emerged as the most effective technique among those considered.[44] Furthermore, multiple studies have shown the heightened bactericidal efficacy achieved by combining chemical irrigation with laser irradiation, as opposed to non-irradiated canals, disinfected solely with irrigants.[5,44,46,47] This improved effectiveness in root canal cleansing can expedite the PL healing process, as demonstrated in our current investigation.

An additional aspect addressed in our current research pertained to postoperative pain, an important concern

Table 2. Mean pain scores on the visual analog scale (VAS), assessed at 1, 2 and 4 days postoperatively in both groups

Time point	G1	G2	*t*	df	*p*-value
After 1 day	4.10 ±1.86	3.11 ±1.29	1.95	38	0.030*
After 2 days	2.95 ±1.39	2.16 ±0.96	2.06	38	0.023*
After 4 days	1.15 ±0.67	0.89 ±0.66	1.20	38	0.119

Data presented as *M* ±*SD*.
Groups: G1 –control group; G2 – test group. * statistically significant (Student's *t* test for independent samples).

in endodontic treatment. The incidence of pain following endodontic treatment has been documented to vary between 3% and 58%.[29,48] Moreover, RCR has been associated with a higher frequency of flare-up pain in comparison with the initial root canal treatment.[49] Low-level laser therapy emerges as a non-pharmacological method capable of expediting cellular processes and averting postoperative pain.[30] Numerous investigations utilizing VAS to gauge pain intensity have indicated that LLLT effectively diminishes postoperative discomfort after both root canal treatment and RCR.[41,42,50] Asnaashari et al. conducted a study demonstrating significantly reduced pain scores within 48 h of treatment.[41] However, they observed limited pain reduction effects from LLLT/PBM irradiation in the context of endodontic retreatment involving mandibular first and second molars.[41] Naseri et al. exhibited a substantially greater pain reduction post-endodontic treatment as compared to a placebo across all study intervals.[50] Moreover, the test group necessitated significantly fewer analgesics than the placebo group. They concluded that LLLT effectively supplemented oral analgesics in mitigating postoperative pain.[50] Nevertheless, findings from Arslan et al. show that while the LLLT group exhibited significantly reduced postoperative pain in the initial 4 days as compared to the placebo group, no statistically significant differences were discerned between the 2 groups on days 5 and 7.[27] This led them to conclude that PBM might alleviate postoperative pain subsequent to RCR in mandibular molars.[27] In our study as well, we observed a notable reduction in the pain levels on the 1st and 2nd days following treatment.

The rationale behind the role of the 635-nm wavelength diode laser in augmenting the healing of PLs is rooted in its ability to enhance cell viability through the stimulation of mitochondrial and cell membrane photoreceptors.[28] This stimulation prompts the synthesis of adenosine triphosphate (ATP), further fostering osteoblast proliferation.[28,50] This makes it a potential component in novel clinical strategies that leverage laser energy to bolster the healing process.[41] In a study by Ng et al., the application of LLLT demonstrated a reduction in pain intensity to some degree, albeit within a limited 4-hour postoperative timeframe.[51] Photobiomodulation has exhibited promising outcomes in facilitating periapical tissue healing.[28] Low-level laser therapy has also shown effectiveness in advancing the healing of both soft and hard tissues following endodontic microsurgery, leading to enhanced bone volume and density, as verified by CBCT imaging.[52] It is important to acknowledge that the current number of studies is constrained, necessitating further research to more definitively establish the efficacy of PBM.[53] Notably, the beneficial clinical effects of PBM have been substantiated on a biochemical level as well. Laser therapy has been shown to elevate prostaglandin levels, imparting anti-inflammatory effects, alongside immunoglobulins and lymphokines that elicit the immune system response.[54,55] Additionally, the documented increase in beta-endorphins, pivotal in analgesia, underscores the impact of the therapy.[56] With promising clinical results and biochemical correlations, the application of PBM emerges as a valuable supplementary approach in post-RCR pain management.[51,56]

Limitations

The primary limitation of this study arises from the restricted number of participants included. The assessment of postoperative pain is inherently subjective, relying on various factors, such as the operator's experience and adherence to clinical protocols. This subjectivity introduces potential variability in the interpretation and reporting of pain outcomes. Additionally, the absence of consideration for canal curvature in the eligibility criteria represents a notable constraint in the study. The study findings may be influenced by variations in the Er:YAG laser parameters, as well as the shape and type of the tip, especially when using an Er:YAG laser from a different manufacturer. Furthermore, within the test group, we examined the synergistic impact of 2 laser wavelengths (2,940 nm and 635 nm) on the healing process. Future research should involve distinct groups to assess the individual effects of each laser wavelength on PL healing, providing a more comprehensive understanding of the therapeutic contribution of each wavelength.

Conclusions

The present study revealed that the application of the 2,940 nm laser wavelength for irrigant activation and a 635-nm diode laser for PBM enhanced a decrease in the PL size after RCR. Moreover, the application of LAI and PBM resulted in a lower level of postoperative pain after secondary root canal treatment. The synergistic effect of both lasers during RCR allows clinicians to gain precise and adequate cleaning of the root canal, and ameliorate the shaping process by removing the debris produced during mechanical instrumentation. Furthermore, PBM added to endodontic treatment together with the Er:YAG laser reduces pain occurring in the first 2 days after root canal filling. The application of the Er:YAG laser and PBM seems to bring additional benefits in comparison with classical endodontic treatment.

Ethics approval and consent to participate

The trial was structured as a randomized and controlled study. Approval was obtained from the Local Ethics Committee at the Regional Medical Chamber (permission No.: 318/KBL), and informed consent was acquired from all the participating subjects in line with the principles of 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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Original paper

Prevalence of human papillomavirus DNA in the saliva of patients with oral lichen planus

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Conflict of interest
When authors are identified as personnel of the International Agency for Research on Cancer (IARC)/World Health Organization (WHO), the authors alone are responsible for the views expressed in this article. These views do not necessarily represent the decisions, policies or views of the IARC/WHO.

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Abstract

Background. Oral lichen planus (OLP) is a chronic inflammatory T cell-mediated disease that is classified by the World Health Organization (WHO) as an oral potentially malignant disorder (OPMD). Despite the heightened interest in the influence of human papillomavirus (HPV) on oral cancer, the overall incidence of HPV affecting patients with OLP remains inconclusive.

Objectives. The study aimed to evaluate the presence of alpha-, beta- and gamma-HPVs in saliva samples collected from 31 OLP patients and 19 control volunteers. This hospital-based study has the advantage of providing comprehensive clinical data and oral health history, along with saliva analysis for HPV in the specific OPMD.

Material and methods. The DNA extracted from saliva samples obtained from patients with a clinical presentation of OLP, classified as ICD-10:L43, was analyzed using HPV type-specific bead-based multiplex genotyping assays to detect 21 mucosal alpha-, 46 beta- and 52 gamma-HPV types.

Results. The most common HPV type was HPV-49, with a statistically significant difference found between the OLP and control groups ($p < 0.05$). There was no statistical correlation between high-risk (HR) HPVs (HPV-16 and HPV-18) and OLP in the studied population. Positive results for the mucosal types of DNA in the saliva were associated with the positive beta and gamma types that were consistently identified in the analyzed biomaterial.

Conclusions. Beta-HPV-49 was significantly more prevalent in the saliva of patients with OLP. There was no relationship between HR HPVs and the OLP status, which represents the difference between OLP studied in this research and other OPMDs.

Keywords: saliva, oncology, oral lichen planus, mouth neoplasms, human papillomavirus

Highlights

- Beta-HPV-49 was the most common human papillomavirus (HPV) type detected in the saliva of patients with oral lichen planus (OLP).
- No association was identified between high-risk HPV types (HPV-16 and HPV-18) and OLP in the studied population.
- When mucosal HPV types were detected in saliva, beta- and gamma-HPV types were consistently present in the same samples.

Introduction

Oral lichen planus (OLP) is a chronic inflammatory T-cell-mediated disease classified by the World Health Organization (WHO) as an oral potentially malignant disorder (OPMD), with an overall incidence of 0.49–1.43% in the general population.[1] Oral lichen planus is a chronic condition[2–4] that is not related to tobacco smoking or betel chewing, two widely discussed risk factors for OPMDs.[5] The possible association of hormonal changes with the development of OLP has been suggested since the disorder exhibited an increased incidence in post- and peri-menopausal non-smoking and non-alcohol-drinking women.[6–8] Other causal links for OLP formation include diabetes mellitus, infection with the hepatitis C virus (HCV) and stress.[6,7] The clinical management of OLP is of great importance, as there are no established programs for primary prevention beyond the avoidance of tobacco and the cessation of alcohol consumption.[2,9] Despite the considerable interest in the influence of different viral infections on OPMDs, it has not been conclusively defined how the presence of different human papillomaviruses (HPVs) affects OLP patients and influences the eventual progression to oral squamous cell carcinoma (OSCC). The diagnostic process of OLP in the dental office is presented in Fig. 1.

Human papillomaviruses are a family of double-stranded DNA oncoviruses that are associated with invasive malignancies. They are divided into 3 genera: *Alphapapillomavirus* (alpha-HPV), which includes 12 mucosal high-risk (HR) HPV types, namely HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, and -59[10–12]; *Betapapillomavirus* (beta-HPV); and *Gammapapillomavirus* (gamma-HPV), predominantly isolated from skin. However, a variety of HPV types may be present in different bodily subsites.[13] In particular, there is a connection at the level of the genital mucosa, the upper respiratory tract and the skin.[14] Mucosal HR alpha-HPV types are considered well-established risk factors for anogenital tract squamous cell carcinoma[15] and head and neck squamous cell carcinomas (HNSCC) in the area of the tonsils and base of the tongue at the oropharynx.[16,17] Mucosal HPVs have been associated with the majority of oropharyngeal squamous cell carcinoma (OPSCC) cases in the United States and other developed countries, and a minority of HNSCC cases have stemmed from palatine tonsils and lingual tonsils.[18,19]

Cutaneous HPV types may also have an influence on carcinogenesis. The epidemiological and biological models support the synergistic cooperation between beta-HPVs and ultraviolet (UV) radiation in this process.[12,20] Beta-HPV infection plays a "hit-and-run" role in the initial phase of skin carcinogenesis, and an optional role for tumor cell growth once they have become malignant.[12,20,21] These types of viruses are subsequently divided into 5 species (beta-1 to beta-5), with the first two including the majority of beta types present in the skin of healthy individuals,[20,22] and beta-2 mostly found in squamous cell carcinomas.[23] However, while the majority of the beta subtypes are found on the skin, there have been indications suggesting a dual tissue tropism for specific HPV types in the oral and nasal mucosa.[20] Interestingly, in vivo and in vitro experimental models have demonstrated that beta-3 HPV-49 shares biological properties with mucosal HR HPV types, as it immortalizes human primary keratinocytes, which serve as the natural host cells for HPVs.[12]

The gamma genus comprises 27 species, yet no studies have evaluated the possible association of this HPV type with human carcinogenesis, except for a functional in vitro

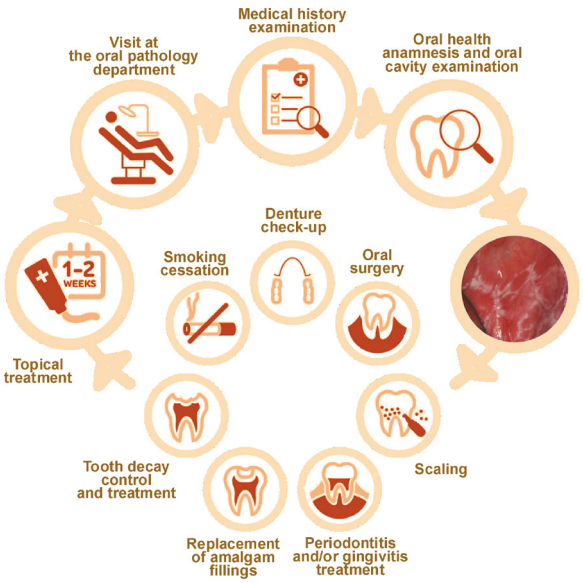

Fig. 1. Schematic overview of the management pathway in the oral pathology department for a patient with an oral lichen planus (OLP) lesion

experimental model that aimed to highlight the transforming properties of gamma-24 HPV-197.[20] While the available data confirms that gamma types can be detected in the anal mucosal epithelium, the results of an extensive epidemiological nested case–control study suggest a broader role for HPVs in HNSCC etiology, as indicated by the associations with gamma-11 and gamma-12 HPV species.[24] The study also suggests that easily collected oral samples could provide a prospective biomarker for the prognosis of HNSCC.[24]

Further investigation is required to elucidate the relationship between OPMDs and HPV infection. In one of the studies, the oral mucosal cells from individuals with OLP presented signs of apoptosis, suggesting similarities to HPV infection.[25] However, the data revealed significant variations.[25–27] Another study suggested that a cooperation between p53 and heat shock protein 90 (HSP90), as well as between HPV-16/18 and HSP90, may affect the biological behavior of OLP. The observed expression of HSP90 and p53 in OLP, as well as the increase in these proteins upon presentation of OSCC, suggests that these factors participate in the malignant transformation of OLP.[28]

Saliva represents a non-invasive biosample for laboratory diagnostics in the oral cavity.[29–32] Previous studies have primarily focused on detecting a limited number of HPV types in OLP, and comprehensive genotyping of all 3 genera in the saliva of OLP patients has not been conducted. Given the differences in the pathophysiology of OLP compared to other OPMDs, we hypothesize that the composition of salivary viruses in patients with OLP may differ. Thus, the present study aimed to evaluate the presence of alpha-, beta- and gamma-HPVs in saliva samples collected from 31 OLP patients and 19 control volunteers using highly sensitive HPV genotyping assays.[33]

Material and methods

Study sample

The study population comprised individuals with a clinical diagnosis of OLP who had agreed to participate in the study. The inclusion criteria were as follows: a histopathological and/or clinical diagnosis of OLP, classified as ICD-10:L43 according to the WHO criteria[34]; and patients enrolled at the Oral Pathology Outpatient Clinic, Department of Oral Pathology, Wroclaw Medical University, Poland, within a 9-month period. No age range was imposed. The exclusion criteria encompassed patients who had undergone any cancer therapies (oral or other) upon admission, as well as those with clinical and histopathological features of oral lichenoid lesions classified as oral lichenoid contact lesions, oral lichenoid drug reactions, and graft-versus-host disease at the time of the research.

The data collected from patients' medical histories after pseudo-anonymization included sociodemographic information (age, sex, place of residence) and medical profiles of the participants. The demographic data analysis was profiled based on patient disclosure (Table 1). The relationship between tobacco consumption and the duration of smoking and/or cessation was considered. Patients were assigned to experimental groups using a simple randomization method.

To provide a clinical evaluation of the control group (19 subjects), volunteers were recruited from the dentists working in the clinic, dental assistants and administrative personnel who agreed to participate in the study. While some individuals in the control group demonstrated higher than average oral hygiene (i.e., dentists), the bias was minimized by the inclusion of administrative workers in the study. The exclusion criteria for the control group consisted of the current diagnosis of OLP or any alterations pertaining to OPMDs and/or periodontitis. The inclusion criteria encompassed a healthy state of the oral cavity, as well as not undergoing any generalized treatment or cancer treatment at the time of saliva collection.

The study was approved by the Bioethics Committee of Wroclaw Medical University, Poland (approval No. KB760/2021). The participants provided written informed consent after receiving information about the project and prior to the collection of biomaterials.

Clinical evaluation of the oral health status

The analysis of the patient's oral health status and clinical OLP presentation was performed by the oral pathology specialist. Histopathological diagnostics were offered to patients with persisting lesions of uncertain clinical features, when there was a progression of the lesion, and for the exclusion or confirmation of epithelial dysplasia. This clinical intervention was selected to minimize unnecessary scarring of the lesion, as discussed by González-Moles et al.[1] Saliva samples were collected from the patients at the beginning of the visit, following the preliminary oral health examination and prior to invasive dental procedures. The protocol for saliva collection has been previously described.[30] Afterward, the salivary samples were transported on dry ice to the Epigenomics and Mechanisms Branch of the International Agency for Research on Cancer (IARC) (Lyon, France) for HPV subtype analysis. The results of the HPV analysis were scored by a laboratory professional who was blinded to the condition and experimental group division.

DNA extraction

The extraction of DNA from saliva was performed using the BioRobot® EZ1 DSP Workstation with the EZ1 DNA tissue kit, in accordance with the manufacturer's instructions (Qiagen, Hilden, Germany). Briefly, following

Table 1. Characteristics of patients with oral lichen planus (OLP), including the distribution of general diseases ($N = 31$)

Variable			Result
Age [years] *M* ±*SD* (range)			62.39 ±11.02 (37–82)
Sex		male	3 (9.68%)
		female	28 (90.32%)
Size of the city of residence		<20.000 inhabitants	9 (29.03%)
		20.000–100.000 inhabitants	10 (32.26%)
		>100.000 inhabitants	12 (38.71%)
Smoking habit		no	30 (96.77%)
		yes	1 (3.23%)
Characteristics of OLP	skin OLP lesions	no	28 (90.32%)
		yes	3 (9.68%)
	clinical erosive form	no	22 (70.97%)
		yes	9 (29.03%)
	mirrored changes (symmetrical)	no	15 (48.39%)
		yes	16 (51.61%)
	histopathological investigation	no	18 (58.06%)
		yes	13 (41.94%)
	histopathological results of erosive and/or high-grade changes	no	25 (80.65%)
		yes	6 (19.35%)
General diseases and disorders	hypertension	no	28 (90.32%)
		yes	3 (9.68%)
	diabetes	no	27 (87.10%)
		yes	4 (12.90%)
	thyroid disorders	no	28 (90.32%)
		yes	3 (9.68%)
	cancer in the history	no	28 (90.32%)
		yes	2 (6.45%)

M – mean; *SD* – standard deviation.

centrifugation at 6,000 rpm for 10 min, the cell pellets were incubated in proteinase K and G2 buffer (Qiagen) at 56°C for 3 h. Subsequently, the DNA was extracted into a 50-µL Eppendorf tube (Eppendorf, Hamburg, Germany).

Human papillomavirus DNA analysis

Molecular diagnostics, namely DNA extraction and HPV DNA genotyping, were performed at the Epigenomics and Mechanisms Branch of the IARC (Lyon, France).

The extracted DNA was analyzed using an HPV type-specific multiplex genotyping test (E7-MPG), as previously reported.[35] This molecular assay integrates multiplex polymerase chain reaction (PCR) and bead-based Luminex technology (Luminex Corporation, Austin, USA). The multiplex type-specific PCR method uses specific primers for the detection of 21 mucosal HR alpha-HPV types (-6, -11, -16, -18, -26, -31, -33, -35, -39, -45, -51, -52, -53, -56, -58, -59, -66, -68, -70, -73, -82), 46 beta-HPVs from β-species 1 (-5, -8, -12, -14, -19, -20, -21, -24, -25, -36, -47, -93, -98, -99, -105, -118, -124, -143, and -152), β-species 2 (-9, -15, -17, -22, -23, -37, -38, -80, -100, -104, -107, -110, -111, -113, -120, -122, -145, -151, -159, and -174), β-species 3 (-49, -75, -76, and -115), β-species 4 (-92), and β-species 5 (-96 and -150); and 52 gamma-HPVs from γ-species 1 (-4, -65, -95, and -173), γ-species 2 (-48 and -200), γ-species 3 (-50), γ-species 4 (-156), γ-species 5 (-60 and -88), γ-species 6 (-101, -103 and -108), γ-species 7 (-109, -123, -134, -149, and -170), γ-species 8 (-112, -119, -164, and -168), γ-species 9 (-116 and -129), γ-species 10 (-121, -130, -133, and -180), γ-species 11 (-126, -169, -171, and -202), γ-species 12 (-127, -132, -148, -165, and -199), γ-species 13 (-128), γ-species 14 (-131), γ-species 15 (-179), γ-species 18 (-156), γ-species 19 (-161, -162 and -166), γ-species 20 (-163), γ-species 21 (-167), γ-species 22 (-172), γ-species 23 (-175), γ-species 24 (-178 and -197), γ-species 25 (-184), and γ-species 27 (-201), as well as SD2.[36] An additional set of primers for β-globin was also included. After PCR amplification, 10 µL of each reaction mixture was analyzed using a Luminex-based assay, as previously described. For each probe, the mean fluorescence intensity (MFI) obtained when no PCR

product was added to the hybridization mixture was considered the background value. The cut-off was computed by adding 5 MFI to ×1.1 of the median background value. All MFI values that exceeded the designated cut-off point were considered positive.[35]

Statistical analysis

The obtained results were statistically analyzed using STATISTICA™ software, v. 13.3 (StatSoft Inc., Tulsa, USA) and the Python Programming Language (RRID:SCR_008394, package Dython, v. 0.7.4; Python Software Foundation, Wilmington, USA). The data was presented as numerical values, percentages, and means with standard deviations. The Shapiro–Wilk test was employed to assess the distribution of the data. The comparisons between the control and patient group variables with a normal distribution were assessed with the use of Student's *t*-test for independent variables. The odds ratio (*OR*), standard error (*SE*) and 95% confidence intervals (*CIs*) were calculated according to Altman.[37] The χ^2 test of independence was used to identify the relationship between categorical variables, and the Phi coefficient or Cramér's V was employed to assess the effect size of the relationship between binary or multicategorical variables, respectively. Pearson's correlation coefficient (*r*) was utilized to ascertain the relationships between continuous variables. The differences were interpreted as statistically significant at $p < 0.05$.

Results

General characteristics and clinical evaluation of the study groups

The demographic and clinical characteristics of the patients are provided in Table 1. The mean age of the participants was 62.39 ±11.02 years, ranging from 37 to 82 years. The majority of the included patients were from cities with a population exceeding 100,000 inhabitants (*n* = 12; 38.71%). Oral lichen planus was diagnosed more frequently in women (*n* = 28; 90.32%) during the ongoing recruitment of patients that persisted for 9 months.

A detailed description of the OLP patients' oral health is provided in Table 2. The individuals diagnosed with OLP presented additional changes to the oral health status, including desquamative gingivitis (*n* = 7; 22.58%), xerostomia (*n* = 2; 6.45%), geographic tongue (*n* = 2; 6.45%), and bruxism (*n* = 2; 6.45%).

A histopathological analysis of any OLP lesions was performed in 13 subjects (41.94%). The pathologist observed erosive and/or high-grade changes in 6 subjects (19.35%). The three most common areas affected by OLP were the cheeks (*n* = 15; 48.39%), cheeks and tongue (*n* = 7; 22.58%), and the molar triangle (*n* = 2; 6.45%).

Table 2. Results of the clinical evaluation of oral mucosal changes in patients diagnosed with oral lichen planus (OLP)

Mucosal changes	OLP group (*n* = 31)
Xerostomia	2 (6.45)
Desquamative gingivitis	7 (22.58)
Oral cancer prior to OLP	1 (3.23)
Burning mouth syndrome	1 (3.23)
Burning mouth syndrome and unrelated white changes on the tongue	1 (3.23)
Burning mouth syndrome and desquamative gingivitis	1 (3.23)
Geographic tongue	2 (6.45)
Bruxism	2 (6.45)
No additional pathological changes on the oral mucosa	16 (51.61)

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

A total of 3 subjects (9.68%) reported a generalized disorder of lichen planus (skin type) and had either consulted with, or had been consulted by, a dermatologist. The most declared general disorder was diabetes, reported by 4 patients from the study group (12.90%).

The volunteers from the control group (*n* = 19) declared no smoking habit or use of any other form of tobacco or vaping systems. The examination of the subjects' oral cavities revealed no significant changes or evidence of OLP or other pathologies.

Additionally, the relationships between questionnaire variables are presented in Fig. 2. A marked relationship was identified between hypertension and geographic tongue, as well as between the manifestation of skin OLP and the presence of burning mouth syndrome in individuals with OLP.

DNA molecular results

HPV genotyping

For all samples, the presence of high-quality DNA was confirmed by positive PCR amplification of the *β-globin* gene, as demonstrated in the supplementary materials (available on request from the corresponding author).

The frequencies were compared using the χ^2 test.[37] Statistically significant values were obtained for the presence of HPV-49 DNA in the saliva of patients from the OLP group when compared to the control group (Table 3). In another part of the analysis, the presence of HPV-17, -24, -47, -104, -105, -113, -143, -4, -121, -123, -130, -134, and -149 was detected in the OLP group but not in the control group, demonstrating HPV-positive OLP.

The well-known oncogenic HPV types 16 and 18 were not detected in the saliva of any patient with OLP.

Regarding the HPV groups, the presence of mucosal types in saliva was accompanied by the detection of beta and gamma types in the analyzed biomaterial. Similarly, with the exception of a single case in the control group, if

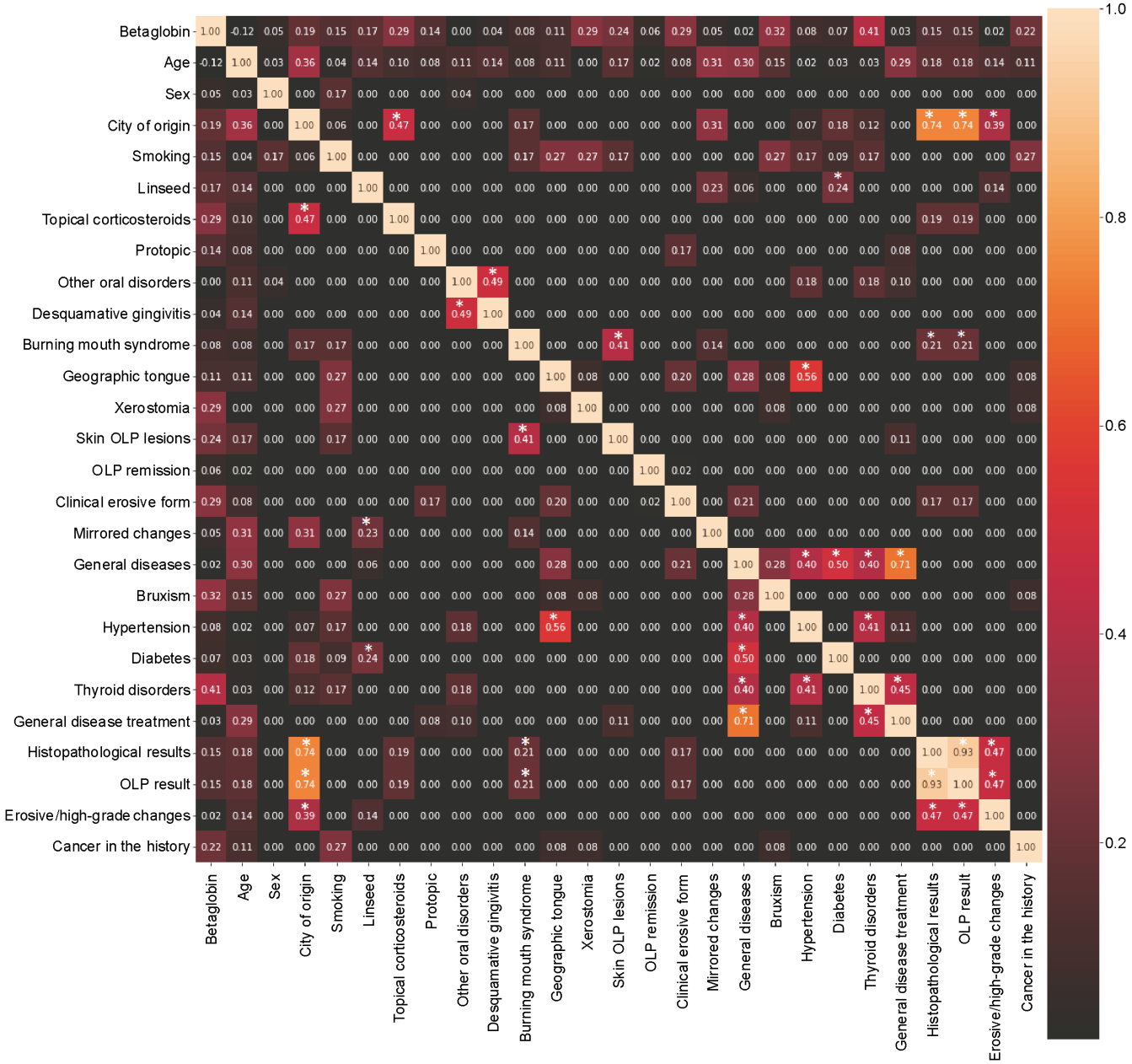

Fig. 2. Matrix of relationship coefficients between variables in the study group

The Phi coefficient and Cramér's V were employed for pairs of nominal variables, Pearson's correlation coefficient for pairs of continuous variables, and the correlation ratio for categorical and continuous variables. The larger the value, the lighter the color, indicating a stronger relationship. Asterisks denote statistically significant values ($p < 0.05$).

a positive beta type result was obtained in saliva, gamma-HPV types were also present (supplementary materials).

As a thorough HPV analysis presented in this manuscript is not widely performed, the table enumerating all positive results for the analyzed HPV subtypes is included in the supplementary materials.

Discussion

In the present study, the clinical and histopathological parameters related to OLP status were evaluated for the presence of specific HPV types. The most prevalent condition observed among OLP patients was desquamative gingivitis, which manifested in 22.58% of the subjects. This disorder is characterized by hypersensitivity to a variety of autoimmune diseases.[38] The majority of patients were non-smoking women (90.32%), and the mean age of the participants was 62 years, which is consistent with previous OLP studies.[39]

The most frequently diagnosed HPV type in this study, which exhibited statistical differences between the OLP and control groups, was beta-HPV-49. In a previous study, it has demonstrated a similar ability to degrade p53

Table 3. Comparison of the frequency of individual human papillomavirus (HPV) types detected in saliva between the control group and the oral lichen planus (OLP) group

HPV type		Control group (n = 19)	OLP group (n = 31)	p-value	*OR* (95% *CI*)
HPV-17	no	19 (100.00)	27 (87.10)	0.10	6.38 (0.32–125.50)
	yes	0 (0.00)	4 (12.90)		
HPV-24	no	19 (100.00)	26 (83.87)	0.07	8.09 (0.42–155.21)
	yes	0 (0.00)	5 (16.13)		
HPV-47	no	19 (100.00)	30 (96.77)	0.43	1.92 (0.07–49.50)
	yes	0 (0.00)	1 (3.23)		
HPV-49	no	18 (94.74)	22 (70.97)	0.04*	7.36 (0.85–63.72)
	yes	1 (5.26)	9 (29.03)		
HPV-104	no	19 (100.00)	29 (93.55)	0.26	3.31 (0.15–72.62)
	yes	0 (0.00)	2 (6.45)		
HPV-105	no	19 (100.00)	27 (87.10)	0.10	6.38 (0.32–125.50)
	yes	0 (0.00)	4 (12.90)		
HPV-113	no	19 (100.00)	30 (96.77)	0.43	1.92 (0.07–49.50)
	yes	0 (0.00)	1 (3.23)		
HPV-143	no	19 (100.00)	29 (93.55)	0.26	3.31 (0.15–72.62)
	yes	0 (0.00)	2 (6.45)		
HPV-4	no	19 (100.00)	29 (93.55)	0.26	3.31 (0.15–72.62)
	yes	0 (0.00)	2 (6.45)		
HPV-121	no	19 (100.00)	29 (93.55)	0.26	3.31 (0.15–72.62)
	yes	0 (0.00)	2 (6.45)		
HPV-123	no	19 (100.00)	30 (96.77)	0.42	1.92 (0.07–49.50)
	yes	0 (0.00)	1 (3.23)		
HPV-130	no	19 (100.00)	28 (90.32)	0.16	4.79 (0.23–98.02)
	yes	0 (0.00)	3 (9.68)		
HPV-134	no	19 (100.00)	28 (90.32)	0.16	4.79 (0.23–98.02)
	yes	0 (0.00)	3 (9.68)		
HPV-149	no	19 (100.00)	29 (93.55)	0.26	3.31 (0.15–72.62)
	yes	0 (0.00)	2 (6.45)		

* statistically significant ($p < 0.05$, Student's t-test); *CI* – confidence interval; *OR* – odds ratio. Data for the control and OLP groups is presented as n (%).

as HPV-16 E6 in an E6AP-dependent mechanism.[40] Beta HPV-49 was reported in 6% of the participants diagnosed with HNSCC and in 4.6% of controls.[41] The HPV-23 subtype was detected in 7 individuals from the study group, with 2 of these patients also being positive for HPV-38. One patient who presented only with HPV-23 has been previously diagnosed and successfully treated for oral cancer, namely OSCC type G1. In a previous study, HPV-23 was identified as the most prevalent type in the oral cavities of healthy individuals.[41]

In the present research, it was hypothesized that patients with OLP may harbor a distinct HPV profile compared to individuals with other OPMDs, potentially altering the contributions to disease evolution. With respect to HPV groups, the presence of mucosal types in saliva was accompanied by the detection of beta and gamma types in the analyzed biomaterial. Similarly, except for 1 case in the control group, when a positive beta result was obtained in saliva, gamma-HPV was also present. These outcomes provide preliminary evidence that HPV, particularly beyond the alpha genus, could be represented in the saliva of patients with OLP. Further longitudinal, extensive cohort studies are essential to determine whether all HPV genera contribute to disease progression and the malignant potential of OLP.

The methodology employed in the most recent medical literature, along with the type of samples used in the analysis, may significantly affect outcomes, especially in studies based on OPMD samples, where viral diagnostics are not standardized. Several case–control studies have shown a higher prevalence of HPV, including mucosal types HPV-16 and HPV-18, in OLP subjects compared to controls.[25] Mohammadi et al. have examined formalin-fixed, paraffin-embedded samples of OLP.[25] Surprisingly, based on these findings, 12 samples (48% of all OLP cases) were found to be positive for HPV-16, compared to 6 samples in the control group. The methodology used by the authors included endpoint PCR and different biomaterials.[25] In contrast, other studies have failed to detect mucosal HPV in OLP,[26,27] suggesting a potential discrepancy in sample quality. In the present study, no correlation was identified between HPV-16 or HPV-18 and the clinical stage, nor were any results for those HR HPV types obtained using highly sensitive Luminex-based assays. A limitation of this study is that the aliquoting process involved a pre-centrifugation step at 2,500 rpm to remove debris, which has presumably reduced the amount of exfoliated oral cells. Nevertheless, DNA has been successfully extracted from all samples, as evidenced by the amplification of the *β-globin* gene.

The available medical literature indicates a paucity of participants in most epidemiological studies on OPMDs, making it difficult to draw definitive conclusions. Most of the analyses of HPV DNA in OLP individuals were performed on a relatively small number of samples (less than 30).[25,26,42] Among the retrospective cohorts, the analyses were conducted on formalin-fixed samples.[25,26,42,43] In this regard, our research, which represents a hospital-based study with an average number of volunteers, has the advantage of providing all clinical data, oral health history and saliva analysis.

Conclusions

Statistically significant values were obtained for the presence of HPV-49 in relation to the frequency of individual HPV types in the OLP group. However, its role in the process of potential progression to OSCC remains to be established.

The investigation revealed no significant correlation between the HR HPV (HPV-16 and HPV-18) and OLP in the studied population.

With regard to the presence of HPV groups, the mucosal types were positively diagnosed in saliva, and the beta and gamma types were consistently identified in the analyzed biomaterial. Similarly, except for a single case in the control group, when a positive beta result was obtained in saliva, gamma-HPV was also present.

Even though saliva samples are easy to collect and safe, this medium is not always a reliable source for detecting viral infections. Tissue samples are more likely to provide reliable results, and in cases of non-healing or severe, advanced oral lichen lesions, the presence of HPV infection is a consistent finding.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee of Wroclaw Medical University, Poland (approval No. KB760/2021). The participants provided written informed consent after receiving information about the project and prior to the collection of biomaterials.

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.

ORCID iDs

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Original paper

Prediction of interactome hub genes in oral cancer and chronic inflammatory periodontitis

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Abstract

Background. Oral infections that cause inflammation typically affect the gingival tissues. The immune-inflammatory reactions significantly influence the patient's vulnerability to periodontal diseases. Numerous studies have found a correlation between persistent inflammation and an increased risk of developing cancer in the afflicted oral epithelium. New research demonstrates a startling connection between periodontal conditions and various forms of cancer, including oral cancer.

Objectives. The aim of the study was to use bioinformatics techniques in order to predict interatomic hub genes in oral cancer and periodontitis.

Material and methods. The datasets were screened for differentially expressed genes (DEGs) in periodontitis and oral cancer using the Gene Expression Omnibus (GEO) database, a gene expression data analysis tool. GeneMANIA was used to identify hub genes between oral cancer and periodontitis. Orange machine learning was conducted for hub gene prediction using random forest, decision tree, AdaBoost, and neural network.

Results. The top 5 hub genes (*RSPO4*, *CDHR2*, *DDAH2*, *HLA-J*, and *IRF3*) were prioritized to explore their relationship with oral cancer and periodontal disease. The receiver operating characteristic (ROC) curve was constructed, with the area under the curve (*AUC*) for random forest at 0.999, for the decision tree at 0.998, for AdaBoost at 1.000, and for the neural network model at 0.865. The AdaBoost model, followed by random forest and decision tree, exhibited the highest level of accuracy (1.000). These results suggest that the 3 models demonstrate good predictability and may facilitate the early detection of periodontitis and oral cancer.

Conclusions. The insights derived from this study may contribute to the development of novel diagnostic and therapeutic techniques for chronic inflammatory periodontitis and oral cancer by utilizing computational approaches and integrating multi-omics data. The identification of interactome hub genes in these diseases has important clinical ramifications. The obtained outcomes may help decipher disease pathways, promote early detection, and create targeted treatments for better patient outcomes. The accurate prediction of hub genes may promote their utilization as biomarkers in the development of individualized treatment plans for both illnesses.

Keywords: hub genes, periodontitis, chronic inflammation, oral cancer, interactome

Highlights

- Five key interactome hub genes (*RSPO4*, *CDHR2*, *DDAH2*, *HLA-J*, *IRF3*) were identified as molecular links between chronic periodontitis and oral cancer.
- Machine-learning models, especially AdaBoost and random forest, showed excellent predictive accuracy ($AUC \approx 1.0$) for distinguishing hub vs. non-hub genes.
- These findings highlight shared immune-inflammatory pathways and support the development of early diagnostic biomarkers and targeted therapeutic strategies for both diseases.

Introduction

Periodontal infections trigger a prolonged immune-inflammatory response that damages oral tissues locally and encourages chronic systemic inflammation, which contributes to cancer etiology.[1] According to the most recent meta-analysis, periodontal disease increases the chance of developing pancreatic, lung, and head and neck malignancies. A recent cohort study with a 10-year follow-up found a substantial link between periodontitis and cancer mortality.[2–4]

Oral carcinogenesis is a multifactorial, intricate process that occurs when several genetic changes impact epithelial cells. A few normal keratinocytes undergo a transformation, initiating the process of oral carcinogenesis. Modifications to cytogenetic and epigenetic processes may influence this shift. These alterations can impact the cell cycle, DNA repair procedures and cell differentiation.[5] When risk factors are combined with these changes, an unstable keratinocyte develops, eventually evolving into a pre-cancerization field and giving rise to malignant neoplastic alterations.

It is estimated that chronic inflammation is the underlying cause of 15–20% of cancers. Based on the 2017 classification,[6] the risk of developing oral cancer has been significantly and independently associated with periodontitis. Studies have also shown a clear causal relationship between the amount and severity of periodontitis,[6] as well as between the amount and severity of chronic periodontitis and the risk of oral cancer, even after controlling for common confounding factors, such as smoking, alcohol use and the human papillomavirus (HPV).[7–11] According to recent research, the loss of teeth due to bone loss in periodontitis is a distinct risk factor for head and neck cancer. Additionally, patients with periodontitis exhibit elevated levels of human telomerase reverse transcriptase, whose expression is particularly specific to cancer cells.[12,13] These findings provide substantial evidence to support a strong connection between periodontitis and oral cancer.[14–23] The available studies suggest a potential link between HPV infection, periodontitis and certain types of cancer. Human papillomavirus is a sexually transmitted infection that can cause various types of cancer, including cervical, anal, and head and neck cancers.

The etiology of both periodontitis and oral cancer is multifactorial, involving complex interactions between genetic and environmental factors. The identification of key genes and their interactions in these diseases can provide valuable insights into their pathogenesis and potential therapeutic targets. In recent years, network-based techniques have emerged as effective tools for understanding complex disorders, facilitating the identification of hub genes within protein–protein interaction (PPI) networks. Changes in gene expression frequently signal changes in physiological processes or the onset of disease. The development of diseases, however, may be linked to the molecular functions, biological processes and signaling pathways present in the genetically encoded products.[24–29] Recently, the interactome analysis has gained popularity as a way to understand biological systems' complicated molecular networks. A molecular interaction network among a cell, tissue or organism is called an interactome. The molecular interactions between proteins, nucleic acids and tiny molecules govern biological processes. Hub genes are essential to the interactome, and they interact extensively with other molecules within the interactome network. Through a network of connections, hub genes control many cellular functions. These genes reveal the functional architecture of cellular pathways and the role of certain genes in biological processes.

Recent clinical and experimental research has focused on the identification of diagnostic indicators. A diagnostic cancer marker may be age-, stage-, tissue-, relapse-, or follow-up-specific and may manifest at any stage of the disease.[30] The examination of hub genes[31,32] facilitates the identification of biological pathways associated with the disease, enhancing the specificity of therapeutic options for the condition. In the present study, the Gene Expression Omnibus (GEO) database was examined to obtain an optimal dataset, identify key genes, and determine appropriate directions for future investigations. This research uses network analysis techniques to predict interactome hub genes in periodontitis and oral cancer.

Material and methods

Gene expression databases

The present study used gene expression databases, including GEO[33] (periodontitis: GSE186882 and oral cancer:

GSE145272; http://www.ncbi.nlm.nih.gov/geo), to examine the expression patterns of the identified genes in periodontitis and oral cancer. These databases provide valuable information about gene expression levels and can help identify genes with consistent and differential expression.

Differential expression analysis

A differential expression analysis was performed using statistical techniques. This analysis compares the expression levels of genes associated with periodontitis and oral cancer, identifying those that demonstrate significant changes in expression.

Network analysis

A gene co-expression network was constructed using GeneMANIA Cytoscape (https://apps.cytoscape.org/apps/genemania).[34–36] Network analysis can facilitate the identification of hub genes based on their connectivity and interactions with other genes within the network.

Analysis of genes with differentially expressed functions

The research on the major differentially expressed genes (DEGs), Gene Ontology (GO) enrichment, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment was carried out using Enrichr (https://maayanlab.cloud/Enrichr).[37–39] Genesets with a false detection rate (FDR) ≤0.05 were considered significantly enriched. A term with a p-value of ≤0.05 and at least 3 genes in its count was deemed significant. After the relevant phrases were grouped into clusters based on commonalities in their membership, the statistically most significant phrase within each cluster was selected to represent the cluster.

Orange machine learning

Orange (https://orangedatamining.com) is an open-source toolkit for data visualization and machine learning.[40] It provides a user-friendly visual programming interface for data analysis, predictive modeling and visualization. Orange streamlines the machine learning process for users of all skill levels by employing various algorithms and data pre-treatment techniques. Orange is a flexible tool for data analysis and predictive modeling activities, as it facilitates the integration with other well-known machine learning libraries (Fig. 1).

The data was split into training and testing portions. Various machine learning algorithms, namely random forest, decision tree, AdaBoost, and neural network were applied for training.

Cross-validation, model scoring and multi-dimensional scaling are all used within the machine-learning workflows implemented in Orange. Orange[40] integrates widely used

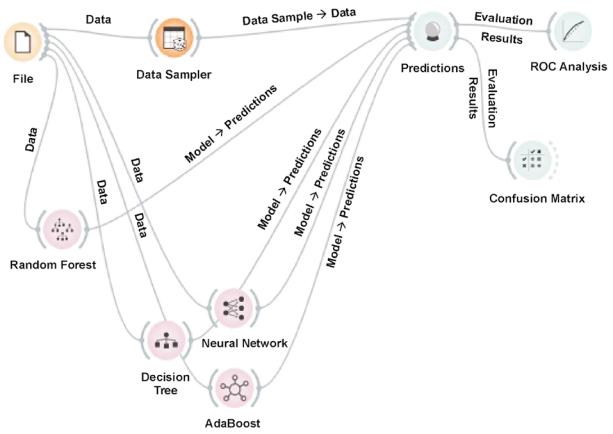

Fig. 1. Orange workflow overview for identifying shared features between periodontitis and oral cancer

ROC – receiver operating characteristic.

Python libraries for data manipulation and machine learning, including NumPy (http://www.numpy.org), SciPy (https://scipy.org) and scikit-learn (https://scikit-learn.org), and encapsulates their functionality within workflow-based building blocks that provide an interface for adjusting machine-learning parameters or browsing results and associated visualizations of inferred models.

Random forest

Random forest is a supervised machine learning algorithm. It can be used for machine learning problems involving both regression and classification. The system learns and specifies the output based on the majority votes of the individual decision trees. Random forest is the most frequently used method for patient classification and biomarker analysis.

Decision tree

Decision trees are a non-parametric supervised learning method for classification and regression. The tree structure is hierarchical, comprising roots, branches, and internal and leaf nodes.

Adaptive boosting

Machine learning ensemble methods use the boosting technique known as the AdaBoost algorithm, sometimes referred to as Adaptive Boosting. The weights are redistributed to each instance, with heavier weights assigned to instances recognized incorrectly, thus explaining the name.

Neural network

Artificial neural networks (ANNs) are modeled after information processing capabilities of organic nervous systems. These networks consist of interconnected neurons that process information and collaborate to solve specific issues.

Results

Differentially expressed genes in periodontitis and oral cancer

Gene expression datasets for periodontitis (GSE186882) and oral cancer (GSE145272) were selected from the GEO database. A total of 500 DEGs were identified using the GEO2R tool from a dataset concerning periodontitis and oral cancer. The cut-off criteria used to define DEGs were |log2 fold change (FC)| >0 and a *p*-value of 0.05. GEO2R is a tool used for the analysis of gene expression data from microarray experiments. It helps researchers identify DEGs between different experimental conditions or groups.

GEO2R uses *t*-tests to compare the means of gene expression levels between different groups or conditions. It calculates the *t*-value and *p*-value for each gene, indicating the significance of differential expression. Additionally, the tool calculates the FC values to determine the magnitude of gene expression differences between groups. The fold change is defined as the ratio of gene expression levels between 2 conditions. These statistical techniques help researchers identify significant DEGs, providing insights into biological processes and potential biomarkers.

Figure 2 depicts a volcano plot of differential gene expression of oral cancer and periodontitis, with red dots representing upregulated genes, blue dots depicting downregulated genes, and gray dots denoting those without substantial differences.

A total of 250 periodontitis genes and 250 genes associated with oral cancer were identified. The top 5 hub genes (*RSPO4*, *CDHR2*, *DDAH2*, *HLA-J*, and *IRF3*) were selected for further study in the context of oral cancer and periodontal disease.

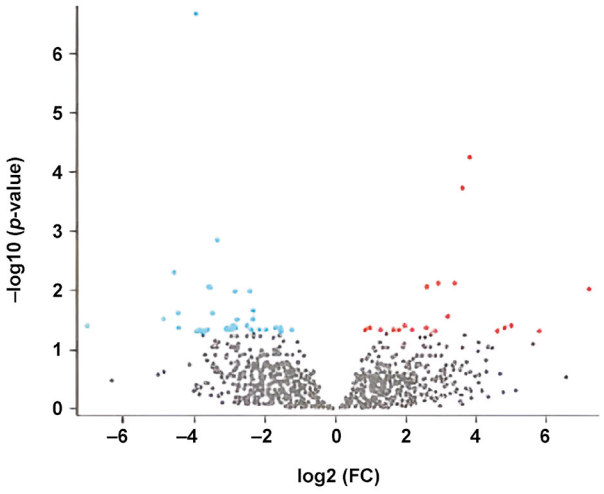

Fig. 2. Volcano plot of differentially expressed genes (DEGs) in oral cancer and periodontitis

FC – fold change. Red dots represent upregulated genes, blue dots depict downregulated genes, and gray dots denote those without substantial differences.

Functional enrichment analysis of DEGs

To further leverage the functionalities of DEGs, gene enrichment analysis was performed. Gene ontology enrichment was carried out using Enrichr. According to the analysis, DEGs were more prevalent in immune-related biological processes, including neutrophil degranulation, neutrophil activation implicated in immune response, and neutrophil-mediated immunity (Fig. 3). The cell cycle mitotic route, epidermal growth factor receptor (EGFR) signaling pathway, transport chain signaling pathway, aurora kinase B signaling pathway, and tumor necrosis factor alpha (TNF-α) signaling via the nuclear factor kappa B (NF-κB) signaling pathway are among the immune-related pathways that comprise the majority of DEGs (Fig. 4A–E). This analysis identified the top 20 clusters with significantly enriched DEGs (Fig. 5).

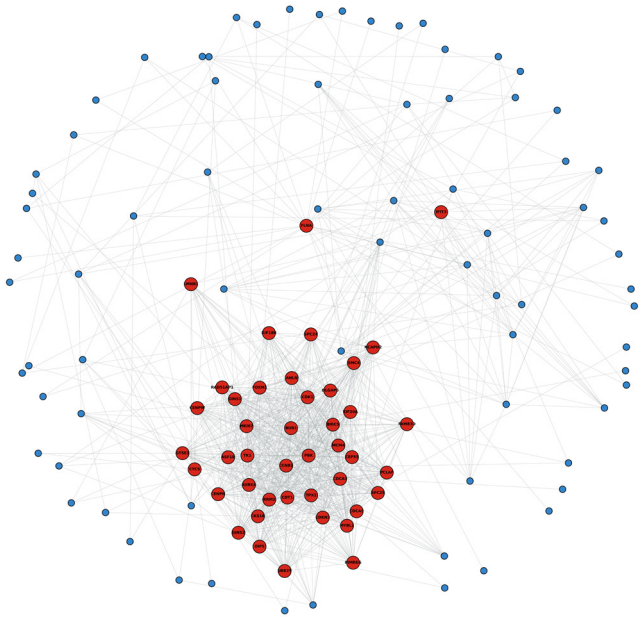

Fig. 3. GeneMANIA network analysis with dense interactions between genes associated with periodontitis and oral cancer

Predictive modeling of hub genes using machine learning

Using the remaining 20% of the testing samples and 80% of the trained samples that were randomly selected for each cross-validation as the training data, we recalculated the similarities between hub and non-hub genes based on the established correlations to evaluate the effectiveness of predicting interactome hub and non-hub genes in periodontitis and oral cancer.

The sample is regarded as affirmative if a hub gene–disease node pair sample demonstrates an observable association and the association score surpasses the established threshold. The true positive rates (TPRs) and false positive rates (FPRs) are primarily calculated to construct

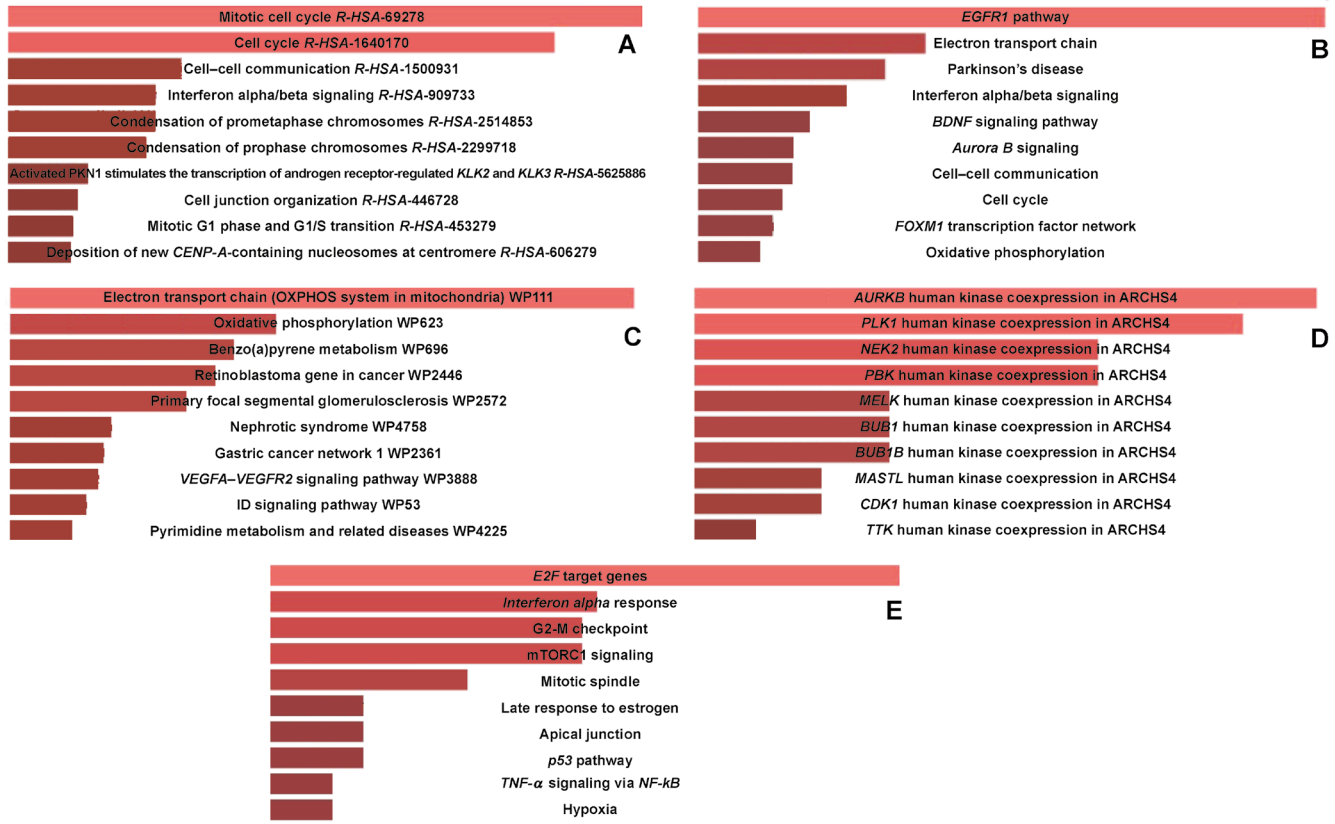

Fig. 4. Gene set enrichment analysis of pathways associated with the cell cycle and mitotic regulation, based on the gene expression profiles

A. Epidermal growth factor receptor (EGFR) signaling pathway; B. Electron transport chain signaling pathway; C. Aurora kinase B signaling pathway; D. Tumor necrosis factor alpha (TNF-α) signaling via the nuclear factor kappa B (NF-κB) pathway; E. Interconnections among key cell-cycle and mitotic-regulation pathways. EGFR – epidermal growth factor receptor; ARCHS4 – all RNA-seq and ChIP-seq sample and signature search.

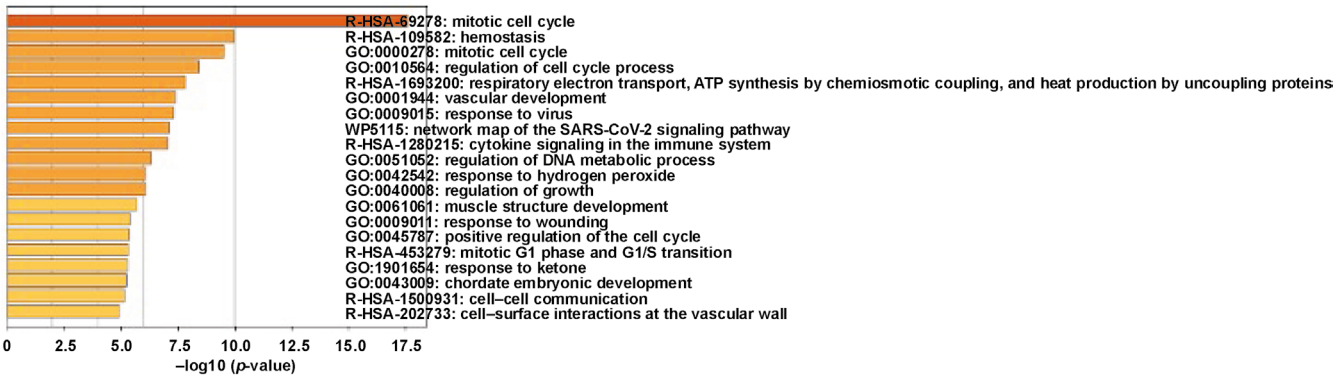

Fig. 5. Bar graph showing the top 20 clusters with significantly enriched differentially expressed genes (DEGs) across the input gene lists

ATP – adenosine triphosphate; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2.

a receiver operating characteristic (ROC) curve. The true positive rate is calculated as follows (Equation 1):

$$TPR = TP/(TP+FN) \quad (1)$$

where:

TP – true positive: samples that were successfully identified and designated as positive;

FN – false negative: the number of hub genes that were incorrectly classified as not belonging to the positive class.

The false positive rate is quantified using the following formula (Equation 2):

$$FPR = FP/(FP+TN) \quad (2)$$

where:

TN – true negative: the number of hub genes correctly identified as not belonging to the positive class.

The ratio of correctly identified negative samples to mistakenly recognized positive samples is expressed as

TN/FP. The area under the receiver operating characteristic (ROC) curve (*AUC*) is a useful indicator of a method's general predictive accuracy (Fig. 6,7).

A significant imbalance was identified between the observed correlations between hub and non-hub genes in diseases (positive cases) and the unobserved correlations (negative cases). In such instances, the effectiveness of a prediction strategy is evaluated using the precision-recall (PR) curve and its area (AUPR). Ideally, the precision parameter for a competent classifier would be set at a value of 1 (high). Precision is defined as 1 when the numerator and denominator are the same, and the FP equals 0 (Equation 3):

$$\text{Precision} = \text{TP}/(\text{TP} + \text{FP}) \quad (3)$$

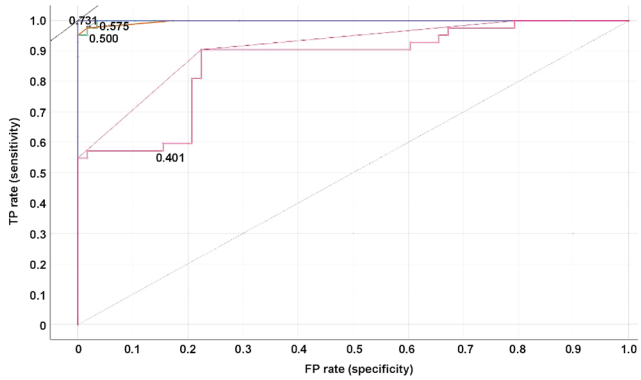

Fig. 6. Receiver operating characteristic (ROC) curve for hub genes, indicating strong predictive performance of the classification model
TP – true positive; FP – false positive.

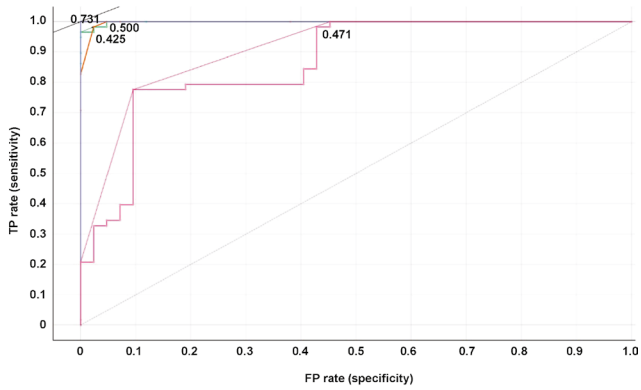

Fig. 7. Receiver operating characteristic (ROC) curve for non-hub genes, indicating strong predictive performance of the classification model

In addition to recall, precision, specificity, and accuracy, a confusion matrix is also used to evaluate *AUC*–ROC curves. The test's target feature is composed of 2 sets of predictions: hub genes and non-hub genes.

The *AUC* values obtained for the various models were as follows: 99% for random forest; 99% for decision tree; 100% for AdaBoost; and 86% for neural network (Table 1).

The evaluation of the predicted results using the confusion matrix for the random forest model produced the classification outcomes for the hub gene (Table 2). Similarly, the confusion matrix results for the decision tree model generated the classification results for the predicted hub gene (Table 3).

The assessment of the AdaBoost model using the confusion matrix showed a TP result of 42 for hub genes and a TN count of 58 for non-hub genes (Table 4).

Finally, the evaluation of the neural network model using the confusion matrix yielded a TP value of 26 for hub genes and a TN value of 74 for non-hub genes (Table 5).

Table 2. Confusion matrix with the random forest model

Variable		Predicted values		
		hub genes	non-hub genes	Σ
Actual values	hub genes	42	0	42
	non-hub genes	2	56	58
	Σ	44	56	100

Table 3. Confusion matrix with the decision tree model

Variable		Predicted values		
		hub genes	non-hub genes	Σ
Actual values	hub genes	41	1	42
	non-hub genes	1	57	58
	Σ	42	58	100

Table 4. Confusion matrix with the AdaBoost model

Variable		Predicted values		
		hub genes	non-hub genes	Σ
Actual values	hub genes	42	0	42
	non-hub genes	0	58	58
	Σ	42	58	100

Table 1. Comparison of model performance across all algorithms

Model	*AUC*	CA	F1	Precision	Recall	Log Loss	Specificity
Random forest	0.999	0.980	0.980	0.981	0.980	0.041	0.986
Decision tree	0.998	0.980	0.980	0.980	0.980	0.055	0.979
AdaBoost	1.000	1.000	1.000	1.000	1.000	0.313	1.000
Neural network	0.865	0.800	0.789	0.827	0.800	0.437	0.737

AUC – area under the receiver operating characteristic (ROC) curve; CA – classification accuracy.

Table 5. Confusion matrix with the neural network model

Variable		Predicted values		
		hub genes	non-hub genes	Σ
Actual values	hub genes	92.3%	24.3%	42
	non-hub genes	7.7%	75.7%	58
	Σ	26	74	100

Discussion

Periodontitis and oral cancer are diseases with distinct etiologies and pathologies. The identification of similar gene signatures in periodontitis and oral cancer can offer useful insights into the shared mechanisms underlying these diseases.[41] Both conditions are associated with persistent inflammation. It has been widely established that oral squamous cell carcinoma (OSCC) is the most prevalent cancer associated with oral bacterial infections. To date, various investigations have revealed the positive effects of *Porphyromonas gingivalis* on the initiation and development of OSCC.[24] Studies have identified several common inflammatory signaling pathways, including the NF-κB route and the mitogen-activated protein kinase (MAPK) pathway. NF-κB regulates gene expression in inflammation, cell survival and the immune response. The activation of the NF-κB pathway has been observed in both periodontitis and oral cancer, contributing to disease development. The MAPK pathway is vital in cell proliferation, differentiation and survival.[24,30,42–44] Its dysregulation has been implicated in both illnesses, causing cellular transformation and tissue damage. Understanding the interactome is crucial for the study of hub genes, as they are highly connected within the interactome network. The identification and characterization of these genes can reveal their functions and potential regulatory mechanisms under various biological conditions. Additionally, the definition of the interactome may vary depending on the specific issue or biological system under investigation.

Epigenetic modifications, such as DNA methylation and histone modifications,[5,45–47] play a significant role in the regulation of gene expression. These changes have linked periodontitis with oral cancer, suggesting the presence of shared underlying processes. Both disorders have been linked to the hypermethylation of tumor suppressor genes, such as p16INK4a (*CDKN2A*), which renders these genes inactive and contributes to the progression of the disease. The deregulation of gene expression in periodontitis and oral cancer has also been linked to histone changes, such as histone acetylation and methylation.

Both periodontitis and oral cancer induce crucial processes involving the immune system and extracellular matrix remodeling. Common gene profiles linked to immune cell infiltration, cytokine generation and matrix remodeling have been identified in various disorders. Interleukin-6 (*IL-6*), interleukin-8 (*IL-8*), and matrix metalloproteinases (*MMP-7*, *-8* and *-9*), for example, are elevated in both periodontitis and oral cancer, which contributes to tissue invasion and destruction. The dysregulated expression of these genes suggests the presence of shared pathways between immune response dysregulation and remodeling of the extracellular matrix. Cytokines, such as IL-6, have been observed to promote the development of tumors by increasing intracellular reactive oxygen species (ROS) and reactive nitrogen intermediates (RNIs), as well as by altering the epigenetic state of certain genes. Additionally, cytokines promote the development of tumors by activating transcription factors that are associated with tumorigenesis. The production of chemokines is then induced by activated transcription factors, leading to ongoing tumor inflammation.

Both periodontitis and oral cancer frequently exhibit changes in tumor suppressor genes and oncogenes. Both disorders are typically characterized by mutations or inactivation of the well-known tumor suppressor gene *TP53*, which promotes uncontrolled cell proliferation and genomic instability. Similar changes have been identified in oral cancer and periodontitis in other tumor suppressor genes, including *CDKN2A*.[48] On the other hand, oncogenes such as *EGFR* and *KRAS* are frequently dysregulated in oral cancer and have also been linked to periodontitis, indicating the existence of overlapping pathways that promote cell proliferation and survival. A network-based analysis has identified several hub genes that are critical for the initiation and propagation of oral cancer. Within PPI networks, these hub genes frequently display significant levels of connection, indicating their crucial role in regulating cellular processes.

In the present study, interactome hub genes associated with periodontitis and oral cancer were identified using datasets from the GEO database. Initially, 20 cases of periodontitis and oral cancer-specific DEGs were found using a variety of machine-learning approaches. The classification of diagnostic models was developed using random forest, decision tree, AdaBoost, and neural networks. According to the ROC curve, the *AUC* for random forest was 0.999, for the decision tree was 0.998, for AdaBoost was 1.000, and the neural network model had an *AUC* of 0.865. The AdaBoost model, followed by random forest and decision tree, exhibited the highest level of accuracy (1.000). The findings indicate that the AdaBoost, random forest and decision tree models have a high diagnostic value and have the potential to facilitate the early detection of periodontitis and oral cancer.[49–53]

The cell cycle mitotic pathway, EGFR signaling pathway, electron transport chain signaling pathway, aurora kinase B signaling pathway, and TNF-α signaling via the NF-κB signaling pathway were all found to have enriched DEGs after the identification of the hub genes. This finding suggests that immune response-related pathways likely play a substantial role in the development of periodontitis and oral cancer.

Network analysis techniques, including gene co-expression and functional interaction networks, have been used to identify interactome hub genes in oral cancer and periodontitis. These methods integrate various data types, such as gene expression patterns and functional annotations, to identify highly connected genes and their associated functional relationships. A total of 250 genes associated with periodontitis and 250 genes related to oral cancer were identified among the 500 DEGs. The top 5 hub genes (*RSPO4*, *CDHR2*, *DDAH2*, *HLA-J*, and *IRF3*) were identified as priority areas for investigation, with a focus on their relationship to oral cancer and periodontal disease.

The gene *RSPO4*,[45] an activator of canonical Wnt signaling, has been linked to stage III–IV, grade C periodontitis in several European populations, which raises the possibility that this gene plays a role in the development of severe, rapid types of periodontitis. Additionally, *RSPO4* regulates interferon-alpha signaling, extracellular matrix interactions, and the mucin barrier.[27]

A unique biomarker for cancer, *CDH2*, can be used to study transendothelial migration and inadequate differentiation. Recent studies suggest that N-cadherin plays a significant role in the pathogenesis of hematologic malignancies, including multiple myeloma and leukemia. The expression of the N-cadherin gene (*CDH2*) is elevated in patients with multiple myeloma who are at high risk for t(4;14)(p16;q32) translocation.[54] Furthermore, increasing the expression of *CDH2* (rs643555C>T) has been connected to biochemical recurrence of prostate cancer and tumor aggressiveness. *CDH2* promotes the epithelial–mesenchymal transition, stemness and metastatic potential of prostate cancer cells by stimulating the ErbB signaling pathway. Additionally, the *DDAH2* gene (chromosome 6p21.3) produces an enzyme that regulates the levels of methyl arginine within cells, thereby facilitating the synthesis of nitric oxide (NO). This, in turn, impedes the activity of nitric oxide synthase (NOS) in healthy cells. A candidate for a hypermethylated gene with downregulated protein expression in OSCC, the *DDAH2* gene, appears to play a crucial role in the development of cancer.

Due to defects in the gene and a lack of any associated functional activity, *HLA-J*, otherwise known as the major histocompatibility complex, class I, J (pseudogene),[55] has long been acknowledged as a pseudogene. However, a recent study found functionally significant transcriptional activity in breast cancer patients.[57] Immunosuppressive proteins HLA-G and HLA-J exhibit a high degree of sequence homology. This provides a starting point for deducing the functional relevance of HLA-J in infection-induced antinociception, particularly in females. According to earlier investigations, IRF3 is functionally implicated in producing cytokines and chemokines in response to the *P. gingivalis* challenge, which leads to the activation of IRF3. The host response to *P. gingivalis* activates IRF3, and IRF3 ablation reduces TNF production in response to *P. gingivalis*.[33–49]

Earlier studies have shown that IRF3 plays a functional role in driving cytokine and chemokine production in response to *P. gingivalis*.[42] Upon exposure to *P. gingivalis*, IRF3 becomes activated. As previously established, the host response to *P. gingivalis* activates IRF3, and IRF3[56] ablation has been demonstrated to reduce TNF production in response to *P. gingivalis*.

The primary objective of future research should be to advance our understanding of the complex interactome networks in periodontitis and oral cancer.[57–59] Integrating multi-omics data, including genomics, trainscriptomics and proteomics will enable a more comprehensive perspective on the underlying mechanisms of the diseases. Collaboration is also necessary to create sizable, well-annotated datasets that can be used for reliable forecasts and validations.

Conclusions

The prediction of interactome hub genes in periodontitis and oral cancer using network-based techniques is a promising direction. The development of targeted treatments and the identification of potential biomarkers would be facilitated by the ability to identify these key genes and their relationships. Further investigation and validation are necessary to fully comprehend the complex molecular networks underlying oral cancer and periodontitis. This knowledge will pave the way for individualized therapies and enhance patient outcomes in the future.

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

The authors used AI-assisted tools (Grammarly, Reverso, ChatGPT, OpenAI, and similar language models) exclusively for language refinement, grammar correction, and enhancing the clarity of writing. No AI tools were used for data analysis, interpretation, statistical procedures, or generation of scientific content. All scientific conclusions were made by the authors.

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Original paper

Identification of salivary volatile organic compounds as the potential diagnostic markers of oral cancer by the gas chromatography–mass spectrometry analysis

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Abstract

Background. Oral cancer (OC) is a major public health problem in the Indian subcontinent. As many as 90% of all OC cases are oral squamous cell carcinomas (OSCCs), often developing from oral potentially malignant disorders (OPMDs). Although the oral cavity is freely accessible, visual identification is often challenging. Biopsy and a microscopic examination is the only confirmatory diagnostic test. Recently, the analysis of volatile organic compounds (VOCs) has emerged as a new, non-invasive, rapid, and inexpensive strategy with promising potential in clinical diagnostics. The human VOCs produced in metabolic pathways, present in body fluids and the exhaled air, can be used for monitoring several oral diseases, including OC.

Objectives. The aim of the present study was to determine the potential diagnostic capabilities of salivary VOCs in OC through identifying and comparing the salivary volatilomic profiles among OSCC and OPMD subjects, as well as healthy controls, using the gas chromatography–mass spectrometry (GC–MS) analysis.

Material and methods. Unstimulated saliva samples were collected from 35 OSCC subjects, 35 OPMD subjects and 40 healthy controls. The VOCs extracted from the ZSM-5/PDMS film were condensed with 100 µL of methanol, of which 1.0 µL was subjected to the GC–MS analysis.

Results. A total of 128 salivary VOCs were detected and identified among the OSCC and OPMD subjects and the healthy controls. Twenty-five metabolites were determined to be statistically significant in differentiating among the 3 groups. Organic acids, alcohols, ketones, alkanes, and acid amides were the major classes of VOCs in the OSCC subjects, while organic acids, alcohols, ketones, acid amides, heterocyclic compounds, and phenols constituted the VOC profile in the OPMD subjects. 1-chloro-dodecane and 1-tridecanol were significant VOCs observed among the controls.

Conclusions. The study demonstrates that salivary VOC profiling can reveal distinct metabolomic alterations in OSCC and OPMDs, with several VOCs emerging as potential tumor-specific biomarkers. While these findings highlight the promise of VOC-based screening, larger studies are needed to validate these markers and establish their clinical applicability.

Keywords: GC–MS, OPMDs, oral cancer, salivary volatile organic compounds (VOCs), metabolomics

Highlights

- A volatile organic compound (VOC)-based metabolomic approach was utilized to profile patients with oral squamous cell carcinoma (OSCC) and oral potentially malignant disorders (OPMDs) in comparison with healthy controls.
- Unique VOC patterns were observed in OSCC and OPMDs, even though both conditions shared similar compound classes.
- The VOC signatures showed significant correlations with key demographic factors, such as age, gender or tobacco use, and clinical factors, like the pTNM and histopathologicl staging.
- Several VOCs have emerged as potential tumor-specific biomarkers for the screening of OSCC and OPMDs, offering promising diagnostic applications.
- Larger, multicenter studies are required to validate these findings and facilitate the clinical implementation of VOC-based screening for OSCC and OPMDs.

Introduction

Head and neck squamous cell carcinoma (HNSCC) represents the 6th most common type of cancer worldwide, with India being considered the global capital for oral cancer (OC), posing a serious health challenge.[1–3] Among HNSCCs, oral squamous cell carcinoma (OSCC) arising from the mucosal surfaces of the oral cavity is the most common type, often preceded by oral potentially malignant disorders (OPMDs), such as oral leukoplakia (OL), oral submucous fibrosis (OSF), oral lichen planus (OLP), etc. Tobacco consumption, including smoked and smokeless tobacco (SLT) products, betel-quid chewing, excessive alcohol consumption, poor oral hygiene, nutrient-deficient diet, and sustained viral infections, e.g., human papillomavirus (HPV), are some of the risk factors associated with the development of OSCC. As compared to the West, the concern with regard to OSCC is significantly higher in India, where about 70% of cases are detected in the advanced stages of the disease, contributing to an increased mortality rate,[4] with the 5-year survival rates around 40%, despite advances in therapy. Hence, early diagnosis and treatment remain the key to improved patient survival.

Most of the research efforts today are focused on the prevention and early detection of the tumor. The visual and screening tests widely used for diagnosis include clinical methods, a cytopathology examination and visualization adjuncts, like tissue autofluorescence. However, the efficacy of these modalities is limited by gaps in the clinician's knowledge and experience, as well as the anatomical site being examined.[5] Although the histopathological examination has remained the gold standard for diagnosing oral dysplastic changes, it is invasive and often tedious to patients; moreover, early lesions of OSCC, including premalignant lesions, are subtle and rarely exhibit the clinical features observed in the established lesions. Hence, there is a high demand for simple, non-invasive and low-cost alternatives, along with a strong unmet need for novel diagnostic tools and prognostic determinants. This underscores the importance of developing new methods for cancer detection.

Some of the approaches include genomics, proteomics and metabolomics. Amongst these, one of the most promising metabolomic approaches is that of volatile organic compounds (VOCs), a family of carbon-containing compounds. Volatile organic compounds cover a range of chemical classes, including aliphatic, aromatic and chlorinated hydrocarbons, aldehydes, ketones, esters, ethers, acids, and alcohols. They are supposed to serve as a potential and specific tool in early cancer detection in breath and body fluids, through the use of various analytical techniques, like gas chromatography–mass spectrometry (GC–MS). Such metabolic profiles act as chemical signatures, capable of characterizing specific processes in the organism, thereby potentially indicating pathologies like OSCC, OPMDs, and other biochemical disorders. During carcinogenesis, there is altered metabolism and upregulated aerobic glycolysis known as the Warburg effect, which induces oxidative stress.[6,7] This liberates highly reactive oxygen species (ROS) that induce the lipid peroxidation of (poly)unsaturated omega-3 and omega-6 fatty acids (PUFAs) in the cellular membranes, mostly generating alkanes and aldehydes as end-products. Considering the high number of hydrocarbons and aldehydes detected in several matrices, this plays a major role in HNSCC and, therefore, the compounds are biomarkers of interest.

Volatile organic compounds are considered potential biomarkers in non-invasive early cancer detection, as screening tools, especially for high-risk patients, e.g., smokers and heavy drinkers, and are also used for post-therapeutic monitoring for recurrence. They have already shown potential as biomarkers for lung, gastric, breast, and prostate cancer, and mesothelioma. Since carcinogenesis is related to inflammation and metabolic changes, VOCs may provide additional diagnostic value as biomarkers for OSCC. However, VOC expression in OSCC has been meagerly reported in the literature, and a major proportion of these studies have analyzed VOC biomarkers in breath. Hence, a substantial gap exists in the use

of salivary VOCs as the potential biomarkers of OSCC in clinical practices.

Saliva contains a wide range of analytes – hydrocarbons, proteins, peptides, hormones, gingival exudates, microbiota, and various small organic metabolites – that exhibit high responsiveness to physiological changes, making it a valuable biofluid for obtaining systemic information. Volatile organic compounds are transferred from blood to saliva, mainly via passive diffusion. Hence, salivary VOCs can reflect m[e]tabolic changes in response to degeneration, inflammation, necrosis, cancer, microbiota alteration, or external factors, such as environmental pollution, medication use and diet.[8] In addition, the use of saliva as a diagnostic tool for OSCC offers several advantages, including the ease of collection, non-invasiveness and cost-effective applicability for screening large populations. Moreover, dysplastic cells from the oral mucosa are continuously shed into saliva due to its close proximity to the epithelial surface. This facilitates the sampling of cells from occult sites, such as the tonsillar crypts, which are otherwise difficult to assess during a routine oral examination.

This study was undertaken to investigate OSCC- and OPMD-associated metabolic adaptations in cells, and to comparatively analyze salivary VOC profiles among OSCC patients, individuals with OPMD and healthy controls by identifying VOC alterations with the use of GC–MS.

Materials and methods

This is a cross-sectional study with 3 groups of participants: OSCC subjects (group 1); OPMD subjects (group 2); and healthy controls (group 3).

Study setting and ethical considerations

The study included OSCC, OPMD and healthy subjects reporting between January 2020 and October 2021 to the Department of Oral Medicine and Radiology and the Department of Surgical Oncology, M.S. Ramaiah Teaching Hospital, Bengaluru, India.

Ethical clearance was obtained from the Ethics Committee for Human Trials of M.S. Ramaiah University of Applied Sciences, Bengaluru, India (No. EC-2020/PG/36). Informed written consent was obtained from patients and controls after explaining the nature of the study. A total of 110 subjects were included in the study: 35 in group 1; 35 in group 2; and 40 in group 3.

Study participants

The inclusion criteria: patients aged >18 years; the histopathological confirmation of OSCC or OPMD; the absence of any treatment at the time of the study, including surgery, chemotherapy or radiation; and good oral hygiene. Age and gender-matched control subjects: without a history of hypertension, diabetes or thyroid disorders, and the use of related medications; without any deleterious habits. The exclusion criteria: subjects with conditions affecting the quantity and quality of saliva (dry mouth, inflammatory and autoimmune salivary gland diseases, etc.).

Sample collection

Unstimulated saliva samples were collected between 8.00 a.m. and 11.00 a.m. (due to the circadian rhythm affecting the salivary flow). The subjects were asked to refrain from using alcohol, tobacco, food, and any oral hygiene products, such as toothpaste and mouthwash, 2 h prior to sample collection. Each subject was asked to thoroughly rinse their mouth with water. Unstimulated saliva (2 mL) was collected in a sterile 10-milliliter glass vial with a screw cap, immediately placed on dry ice in a cold storage box, and stored at –20°C until the pre-extraction process was performed. The collected samples were transported using the cold storage box to Wipro Food and Drug Laboratory, Bengaluru, India, for further processing and analysis. The process was repeated until a sample size of 100 was achieved.

Preparation of the ZSM 5/PDMS hybrid film

For the fabrication of the ZSM-5/PDMS hybrid film, a 50-milliliter glass bottle was used as the supporting substrate. The PDMS solution was prepared by mixing the base and the curing agent in a 10:1 ratio, and then blending it with ZSM-5 to obtain a 20 wt.% ZSM-5 mixture within the PDMS matrix prior to solidification. One gram of this mixture was placed in the glass bottle and kept at 25°C for 3 h, followed by heating at 100°C for 1 h. The resulting sample vials containing the ZSM-5/PDMS film were thoroughly rinsed with methanol and shaken at 120 rpm for 3 days to remove any unreacted PDMS monomers (Fig. 1).

Sample analysis

The 2 mL of the collected saliva sample was diluted with 1 mL of deionized water and added to the ZSM 5/PDMS hybrid film. The VOCs present in saliva were then extracted using a rotary shaker with a variable speed of 120 rpm by vigorously shaking the glass extraction bottle. The extraction container was carefully cleaned with pure water before being dried with liquid nitrogen. Finally, the VOCs extracted from the ZSM-5/PDMS film were condensed with 100 µL of methanol, of which 1.0 µL was subjected to the GC–MS analysis using a GC–MS machine (GCMS-QP2020 NX; Shimadzu, Kyoto, Japan) (Fig. 2).

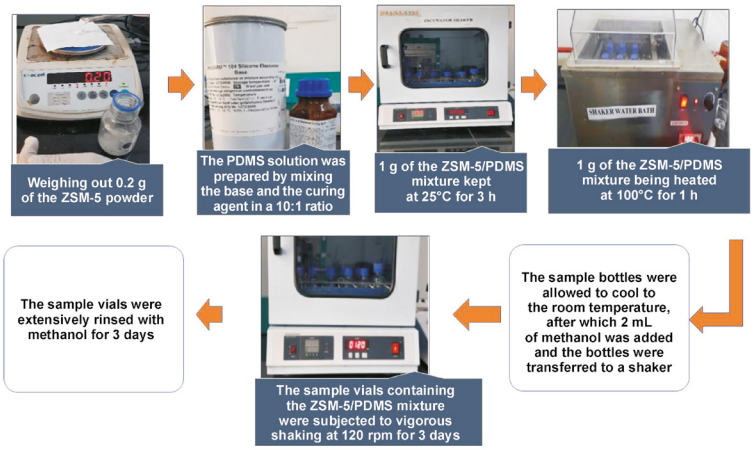


Fig. 1. Steps in pre-extraction – preparation of the ZSM-5/PDMS film

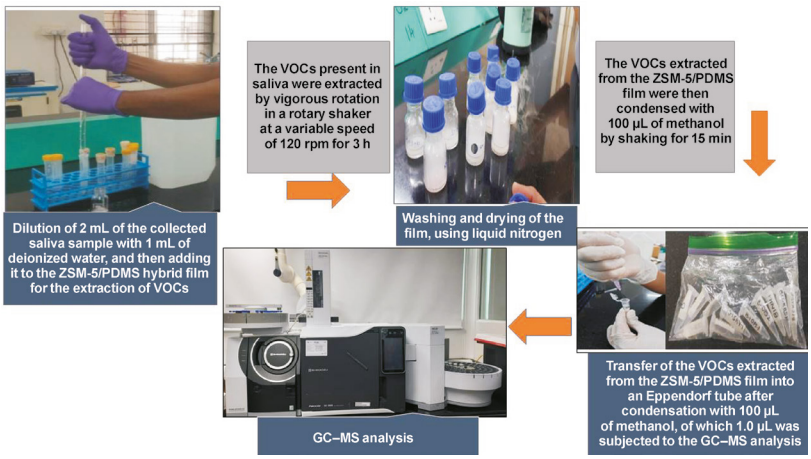
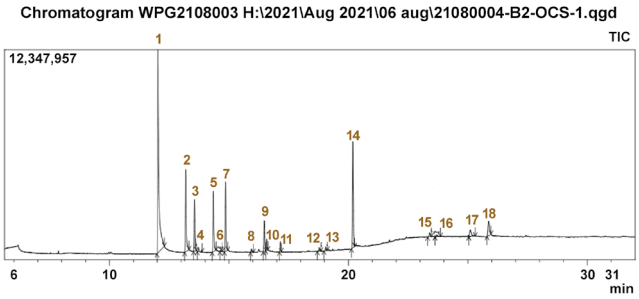


Fig. 2. Pre-extraction followed by the gas chromatography–mass spectrometry (GC–MS) analysis
VOC – volatile organic compound.

GC–MS conditions

The GC–MS machine was used for the chromatographic analysis with mass spectrometric detection in the electron ionization mode at 70 eV. The interphase temperatures of the GC ion source were kept at 200°C and 230°C, respectively. Separation was accomplished using the HP-INNOWax capillary column (polyethylene glycol-based high-polarity stationary phase, length of 30 m, an inner diameter of 0.320 mm, film thickness of 0.25 m; 19091N-113I; Agilent, Santa Clara, USA). The mobile phase was 99.99% ultrahigh-quality helium gas, flowing at 1.78 mL/min. The temperature of the GC injection was fixed at 230°C. The oven temperature was set at 40°C for 3 min, then raised to 230°C at a rate of 10°C per minute for 5 min. The retention time data was recorded from 5 min 6 s to 27 min. Data acquisition was performed in the full scan mode with m/z = 35–500 and a scan time of 0.3 s. The mass spectrum library search program from the National Institute of Standards and Technology (NIST) (v. 2.3) was used to identify the sample chemicals. Compounds having a structural similarity score of over 700 were chosen as the validating biomarkers for successful VOC detection (Fig. 3).



Peak report TIC

Peak No.	Retention time [min]	Area [a.u.]	Area [%]	Compound name
1.	12.027	42,520,515	40.40	acetic acid
2.	13.188	11,976,599	11.38	propanoic acid
3.	13.557	5,738,929	5.45	propanoic acid, 2-methyl-
4.	13.731	396,513	0.38	propylene glycol
5.	14.345	8,125,073	7.72	butanoic acid
6.	14.655	239,855	0.23	3-furanmethanol
7.	14.858	8,637,670	8.21	butanoic acid, 3-methyl-
8.	15.940	529,493	0.50	acetamide
9.	16.478	4,488,550	4.26	pentanoic acid, 4-methyl-
10.	16.580	1,369,028	1.30	butane, 1,4-bis(9,10-dihydro-9-methylanthracen-10-yl)-
11.	17.150	1,012,054	0.96	butane, 1,4-bis(9,10-dihydro-9-methylanthracen-10-yl)-
12.	18.784	393,645	0.37	phosphonic acid, (p-hydroxyphenyl)-
13.	19.047	324,899	0.31	2,5-dimethylfuran-3,4(2H,5H)-dione
14.	20.188	13,939,313	13.24	2-piperidinone
15.	23.400	600,490	0.57	indole
16.	23.687	361,406	0.34	succinimide
17.	25.105	901,064	0.86	benzeneacetic acid
18.	25.875	3,700,728	3.52	hydrocinnamic acid
Total	–	105,255,824	100.00	–

Fig. 3. Representative gas chromatography–mass spectrometry (GC–MS) total ion chromatograms (TICs) of the salivary volatile organic compound (VOC) profile

Results

A total of 110 subjects participated in the study, including 35 OSCC subjects (group 1), 35 OPMD subjects (group 2) and 40 healthy controls (group 3). The demographic characteristics of the subjects are summarized in Table 1.

Group 1 comprised 35 OSCC subjects: stage I – 4 (11.4%); stage II – 2 (5.7%); stage III –16 (45.7%); and stage IV – 13 (37.1%). With regard to the histopathological diagnosis, OSCC was well differentiated in 10 cases (28.6%), moderately in 20 (57.1%) and poorly in 5 subjects (14.3%). The buccal mucosa (both right and left) was the most common site of OSCC, accounting for 13 cases (37.1%), followed by the base and lateral border of the tongue in 8 (22.9%), the gingivobuccal sulcus in 4 (11.4%), the alveolus in 4 (11.4%), the retromolar trigone area in 3 (8.6%), the soft palate in 2 subjects (5.7%), and the upper lip in 1 patient (2.9%) (Table 2).

Group 2 comprised 35 OPMD subjects, including 12 (34.3%) with OL (6 homogeneous and 6 non-homogenous OL cases), 14 (40.0%) with OSF and 9 (25.7%) with OLP. Most of the OL patients had a clinical staging of C1L1P1 – 4 (33.3%) and C2L1P1 – 4 (33.3%). Among the OSF patients, 6 (42.9%) were in grade 2 and grade 3 (in total 12), with 2 (14.3%) in grade 1 and no subjects in grade 4. The REU staging in OLP patients showed a varied distribution (Table 2).

Sample information

The GC–MS analysis of 110 salivary samples using the ZSM-5/PDMS hybrid film detected about 128 distinct VOCs. Amongst them, 25 VOCs were determined to be statistically significant in differentiating between the 3 groups. The major chemical classes of VOCs identified were organic acids (40%), alcohols (20%), ketones (16%), alkanes (8%), acid amides (8%), heterocyclic compounds (4%), and phenols (4%) (Fig. 4). The salivary VOC metabolomic profiles were further evaluated based on gender, age, the type of tobacco used, the pathological tumor (T), node (N) and metastasis (M) (pTNM) classification, and histopathological staging.

Table 1. Demographic characteristics of the study subjects ($N = 110$)

Characteristic		*n*	Group 1 ($n = 35$)	Group 2 ($n = 35$)	Group 3 ($n = 40$)
Gender	M	58	13 (37.1)	25 (71.4)	20 (50.0)
	F	52	22 (62.9)	10 (28.6)	20 (50.0)
Age [years]	21–30	13	1 (2.9)	2 (5.7)	10 (25.0)
	31–40	26	5 (14.3)	8 (22.8)	13 (32.5)
	41–50	34	10 (28.6)	10 (28.6)	14 (35.0)
	51–60	20	7 (20.0)	10 (28.6)	3 (7.5)
	61–70	17	12 (34.3)	5 (14.3)	0 (0.0)
Tobacco use (both smoked and SLT products)	current users	35	10 (28.6)	25 (71.4)	0 (0.0)
	ex-users	18	16 (45.7)	2 (5.7)	0 (0.0)
	non-users	57	9 (25.7)	8 (22.8)	40 (100)
Type of tobacco products used	smoked	5	4 (15.4)	1 (3.7)	–
	SLT	48	22 (84.6)	26 (96.3)	–
Habit duration [years]	1–10	10	6 (23.1)	4 (14.8)	–
	11–20	10	5 (19.2)	5 (18.5)	–
	21–30	27	9 (34.6)	18 (66.7)	–
	31–40	5	5 (19.2)	0 (0.0)	–
	41–50	1	1 (3.8)	0 (0.0)	–
Frequency of use [times/day]	2–4	30	14 (53.8)	16 (59.3)	–
	5–7	20	10 (38.5)	10 (37.0)	–
	8–10	3	2 (7.7)	1 (3.7)	–
Other habits	alcohol consumption	25	15 (42.9)	10 (28.6)	0 (0.0)
Comorbidities	hypertension	14	6 (17.1)	8 (22.9)	–
	diabetes mellitus	8	4 (11.4)	4 (11.4)	–
	both	7	3 (8.6)	4 (11.4)	–
	other	6	4 (11.4)	2 (5.7)	–
	none	35	18 (51.4)	17 (48.6)	–

Data presented as number (percentage) (*n* (%)).
Groups: 1 – oral squamous cell carcinoma (OSCC) subjects; 2 – oral potentially malignant disorder (OPMD) subjects; 3 – healthy controls. SLT – smokeless tobacco; M – male; F – female.

Table 2. Clinical data of the study subjects ($N = 110$)

Data			*n*	Group 1 ($n = 35$)	Group 2 ($n = 35$)	Group 3 ($n = 40$)
Diagnosis	OSCC		35	35 (100.0)	–	–
	OL		12	–	12 (34.3)	–
	OSF		14	–	14 (40.0)	–
	OLP		9	–	9 (25.7)	–
Staging	OSCC pTNM – AJCC (2017)	stage I	4	4 (11.4)	–	–
		stage II	2	2 (5.7)	–	–
		stage III	16	16 (45.7)	–	–
		stage IV	13	13 (37.1)	–	–
	OL[9]	C1L1P0	2	–	2 (16.7)	–
		C1L1P1	4	–	4 (33.3)	–
		C1L2P1	1	–	1 (8.3)	–
		C2L1P0	1	–	1 (8.3)	–
		C2L1P1	4	–	4 (33.3)	–
	OSF[10]	grade 1	2	–	2 (14.3)	–
		grade 2	6	–	6 (42.9)	–
		grade 3	6	–	6 (42.9)	–
		grade 4	0	–	0 (0.0)	–
	OLP[11] (REU staging)	R1E3U0	1	–	1 (11.1)	–
		R1E3U3	1	–	1 (11.1)	–
		R1E6U0	1	–	1 (11.1)	–
		R1E6U3	1	–	1 (11.1)	–
		R1E6U6	1	–	1 (11.1)	–
		R1E6U9	1	–	1 (11.1)	–
		R2E3U0	1	–	1 (11.1)	–
		R2E6U1	1	–	1 (11.1)	–
		R6E8U5	1	–	1 (11.1)	–
Histopathological diagnosis (OSCC)	well differentiated		10	10 (28.6)	–	–
	moderately differentiated		20	20 (57.1)	–	–
	poorly differentiated		5	5 (14.3)	–	–
Site of OSCC	buccal mucosa (right and left)		13	13 (37.1)	–	–
	base and lateral border of the tongue		8	8 (22.9)	–	–
	gingivobuccal sulcus (right)		4	4 (11.4)	–	–
	alveolus		4	4 (11.4)	–	–
	retromolar trigone (left)		3	3 (8.6)	–	–
	soft palate		2	2 (5.7)	–	–
	lip (upper)		1	1 (2.9)	–	–

Data presented as *n* (%).
Groups: 1 – oral squamous cell carcinoma (OSCC) subjects; 2 – oral potentially malignant disorder (OPMD) subjects; 3 – healthy controls. OL– oral leukoplakia; OSF – oral submucous fibrosis; OLP – oral lichen planus; pTNM classification – pathological tumor (T), node (N) and metastasis (M); AJCC – American Joint Committee on Cancer.

Statistical analysis

VOC profiles based on gender

In groups 1, 2 and 3, the VOC profiles were compared according to gender. Six metabolites were statistically significant ($p \leq 0.05$) in group 1, with 5 being more common in males than females. Organic acids (50.0%), alcohols (16.6%), ketones (16.6%), and acid amides (16.6%) were the chemical classes of VOCs identified in the OSCC subjects (Fig. 5). Group 2 showed 3 VOCs that were statistically significantly more common in females as compared to males. Organic acids (100.0%) were the only VOCs found in group 2 (Fig. 6). There were no significant differences in VOCs between genders in group 3.

VOC profiles based on age

Upon comparing the VOC profiles between different age groups, group 1 revealed 3 statistically significant classes of compounds, i.e., organic acids (33.3%), alcohols (33.3%) and ketones (33.3%), which were all more common in the 61–70 age group (Fig. 7), whereas group 2 also revealed 3 significant compounds, i.e., organic acids (33.3%), phenols (33.3%) and monosaccharides (33.3%), which were higher in the 41–50-year and 51–60-year age groups (Fig. 8). However, in group 3, no significant differences in VOCs were found between the age groups.

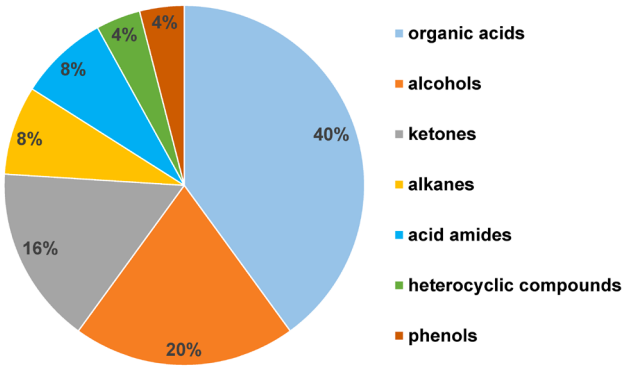


Fig. 4. Relative distribution of volatile organic compounds (VOCs) according to the chemical class

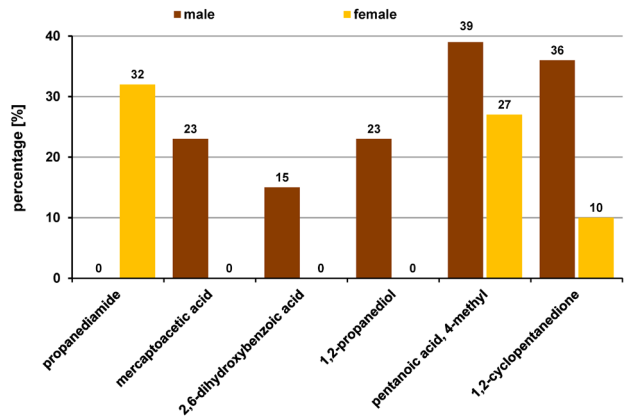


Fig. 5. Distribution of volatile organic compounds (VOCs) based on gender in oral squamous cell carcinoma (OSCC) subjects

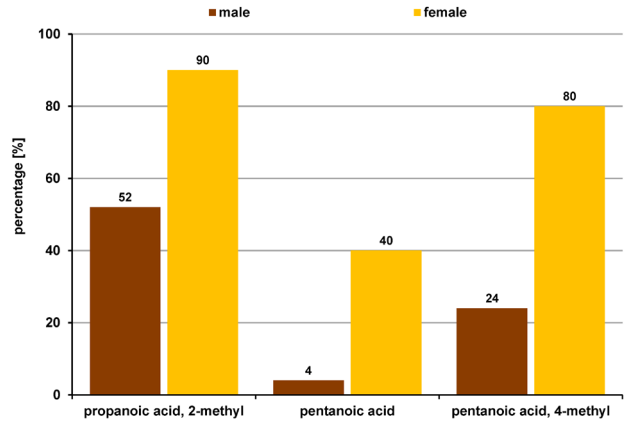


Fig. 6. Distribution of volatile organic compounds (VOCs) based on gender in oral potentially malignant disorder (OPMD) subjects

VOC profiles based on the type of tobacco used

The VOC profiles of the users of smoked and SLT products were compared, and 15 compounds were found to be statistically significant ($p \leq 0.05$), with organic acids (75.0%) and esters (25.0%) being most prevalent in SLT users. Smokers had a mixture of organic acids (54.5%), alcohols (18.1%), amines (9.0%), ethers (9.0%), and nitriles (9.0%) (Fig. 9).

Comparison of VOCs based on pTNM staging in group 1 (OSCC)

When comparing the VOC profiles based on the pTNM staging, 12 VOCs were determined as statistically significant ($p \leq 0.05$). Organic acids, ketones, acid amides, ethers, and aldehydes were the predominant classes of the VOCs identified (Fig. 10).

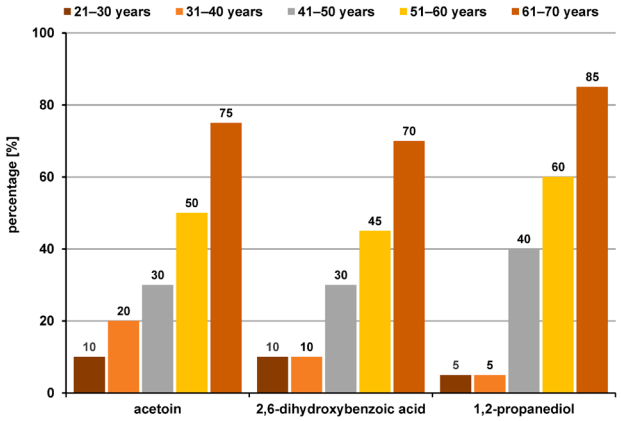


Fig. 7. Distribution of volatile organic compounds (VOCs) based on age in oral squamous cell carcinoma (OSCC) subjects

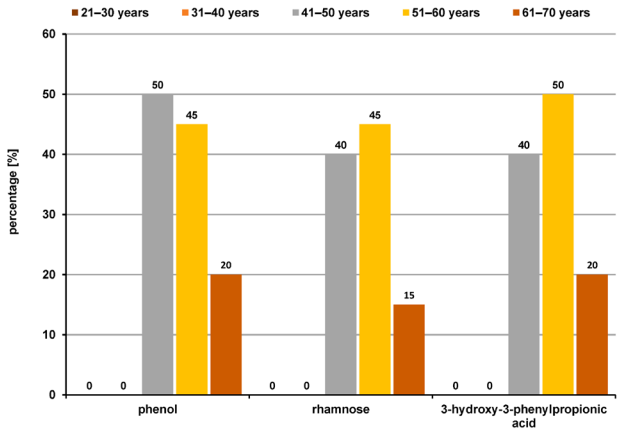


Fig. 8. Distribution of volatile organic compounds (VOCs) based on age in oral potentially malignant disorder (OPMD) subjects

Comparison of VOCs based on histopathological staging in group 1 (OSCC)

Examining the VOC profiles with regard to the 3 histological types of OSCC, 11 VOCs were found to be statistically significant ($p \leq 0.05$; odds ratio (OR) ≥1). Organic acids, ketones, acid amides, ethers, and aldehydes were identified as the predominant classes of VOCs (Fig. 11).

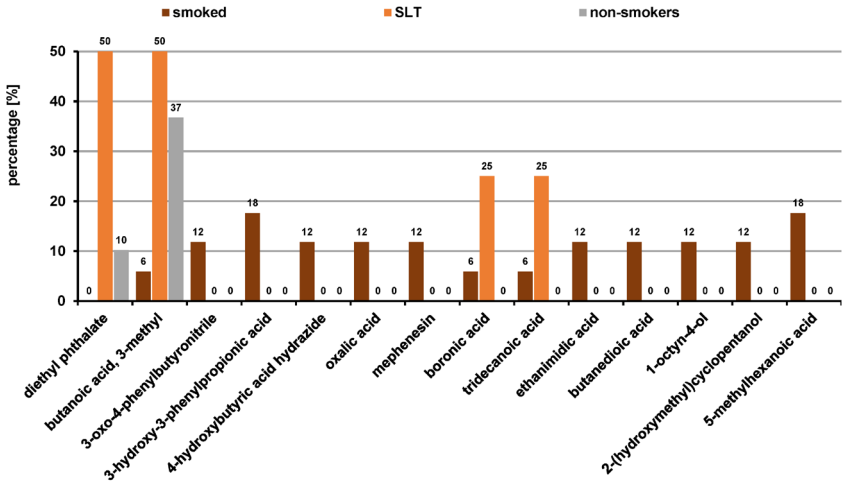


Fig. 9. Distribution of volatile organic compounds (VOCs) based on the type of tobacco used

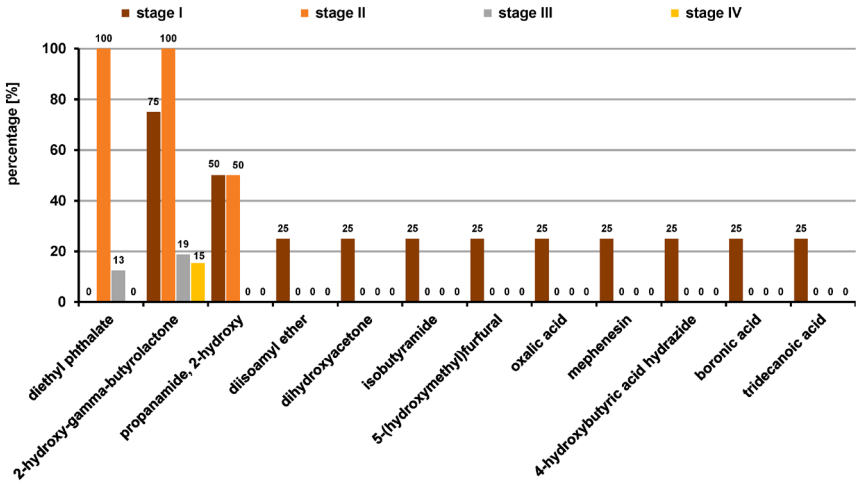


Fig. 10. Distribution of volatile organic compounds (VOCs) based on the pTNM staging in oral squamous cell carcinoma (OSCC) subjects

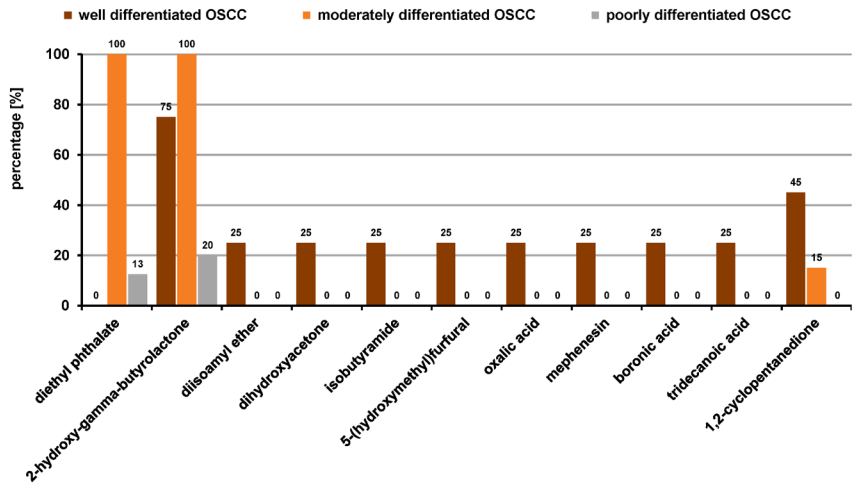


Fig. 11. Distribution of volatile organic compounds (VOCs) based on the histopathological staging in oral squamous cell carcinoma (OSCC) subjects

Discussion

The present study investigated the human salivary volatile metabolome, which might prove to be an accurate and non-invasive tool for distinguishing between OSCC and OPMD subjects, and healthy controls. The GC–MS analysis revealed 128 different VOCs. Amongst the 25 significant VOCs identified, the predominant chemical classes were organic acids (40%), alcohols (20%), ketones (16%), alkanes (8%), acid amides (8%), heterocyclic compounds (4%), and phenols (4%) (Fig. 4). These analytes are clearly products of metabolic processes, i.e., they are endogenous metabolites that remain unaffected by daily activities or environmental conditions.

Although many volatile compounds might be easily detected in salivary samples, they are still influenced by various confounding factors, such as gender, age, diet, smoking, and certain medications. Also, a large number of VOCs in healthy subjects originate from the breakdown of food by normal intestinal flora and they are not indicative of any disease. The VOC profile may vary depending on gender, age, diet, physiological and nutritional status, and habits (alcohol consumption or smoking); therefore, VOCs could be considered as the "odor fingerprint" of individuals.[12] In the present study, the OSCC group revealed more males with statistically significant levels of organic acids, alcohols and ketones associated with oxidative stress (Fig. 5). The fact that OSCC is more frequent in men than women, largely owing to their higher intake of alcohol and tobacco use, justified the higher level of these metabolites in males. In contrast to the VOC profile in the OSCC group, the OPMD subjects showed 3 significant organic acids with statistically significantly higher levels in females than males (Fig. 6). This finding is in accordance with a systematic review by Jia et al., which suggested that gender also influenced the breath VOC profile, with alkenes like isoprene and several other VOCs found to be gender-specific.[12] However, another study by Dragonieri et al. concluded that gender and age did not seem to affect the overall profile of the exhaled VOCs measured by an e-nose device.[13]

Metabolic reprogramming in OC is not yet fully elucidated, and therefore, the investigation of metabolic alterations is crucial for detecting novel diagnostic biomarkers and understanding the disease progression. In a couple of studies, saliva was used to unveil the metabolomic signature of OSCC. Studies have sought to identify salivary metabolite biomarkers that would discriminate OSCC subjects from healthy controls.[14] In the present study, the salivary VOC profiles obtained from individuals with OSCC and OPMDs were compared with regard to each other and healthy controls. Twenty-five VOCs were found significant between the OSCC subjects, the OPMD subjects and the healthy controls (Tables 3–6).

Table 3. Distribution of volatile organic compounds (VOCs) among the oral squamous cell carcinoma (OSCC), oral potentially malignant disorder (OPMD) and healthy subjects (based on the *p*-value)

No.	VOC class	VOCs	Group 1	Group 2	Group 3	*p*-value
1.	organic acids	butanoic acid	77.10%	74.3%	25.0%	0.001*
		pentanoic acid, 4-methyl-	40.0%	20.0%	2.5%	0.001*
		formic acid	11.4%	0.0%	2.5%	0.053*
		benzeneacetic acid	11.4%	20.0%	0.0%	0.015*
		phosphonic acid	20.0%	0.0%	12.5%	0.025*
		mercaptoacetic acid	8.6%	28.6%	10.0%	0.034*
		2-butenoic acid	0.0%	8.6%	0.0%	0.037*
		5-methylhexanoic acid	0.0%	8.6%	0.0%	0.037*
2.	alcohols	1,2-propanediol	8.6%	0.0%	0.0%	0.037*
		1-tridecanol	0.0%	0.0%	15.0%	0.004*
		propylene glycol	20.0%	28.6%	0.0%	0.002*
		3-furanmethanol	14.3%	8.6%	0.0%	0.056*
3.	ketones	2-propanone, 1-hydroxy-	48.6%	25.7%	2.5%	0.001*
		2-hydroxy-gamma-butyrolactone	28.6%	14.3%	2.5%	0.006*
		1,2-cyclopentanedione	20.0%	8.6%	0.0%	0.010*
		acetoin	34.3%	54.3%	10.0%	0.001*
4.	alkanes	butane, 1,4-bis(9,10-dihydro-9-methylanthracen-10-yl)-	11.4%	0.0%	2.5%	0.053*
		1-chloro-dodecane	0.0%	8.6%	30.0%	0.001*
5.	acid amides	propanamide, 2-hydroxy-	8.6%	0.0%	0.0%	0.037*
6.	heterocyclic compounds	indole	2.9%	14.3%	0.0%	0.018*
7.	phenols	phenol	11.4%	40.0%	5.0%	0.001*

Groups: 1 – oral squamous cell carcinoma (OSCC) subjects; 2 – oral potentially malignant disorder (OPMD) subjects; 3 – healthy controls. * statistically significant; red – significant VOCs in the OSCC patients; blue – significant VOCs in the OPMD patients; green – significant VOCs in the healthy controls.

Table 4. Distribution of volatile organic compounds (VOCs) among the oral squamous cell carcinoma (OSCC) and oral potentially malignant disorder (OPMD) subjects (based on the *p*-value and the odds ratio (*OR*))

No.	VOC class	VOCs	Group 1	Group 2	*OR*	*p*-value
1.	organic acids	butanoic acid	77.10%	74.3%	0.780	1.168
		phosphonic acid	20.0%	0.0%	0.000	0.005*
		mercaptoacetic acid	8.6%	28.6%	0.234	0.031*
		formic acid	11.4%	0.0%	0.000	0.039*
2.	alcohols	2-furanmethanol	25.7%	14.3%	2.077	0.232
		3-furanmethanol	14.3%	8.6%	1.778	0.452
3.	ketones	**2-propanone, 1-hydroxy-**	48.6%	25.7%	2.728	0.048*
		2-hydroxy-gamma-butyrolactone	28.6%	14.3%	2.400	0.145
		2,5-dimethylfuran-3,4(2H,5H)-dione	28.6%	25.7%	1.156	0.788
		1,2-cyclopentanedione	20.0%	8.6%	2.667	0.172
4.	alkanes	**butane, 1,4-bis(9,10-dihydro-9-methylanthracen-10-yl)-**	11.4%	0.0%	4.242	0.039*
5.	acid amides	propanediamide	20.0%	14.3%	1.500	0.526
6.	phenols	**phenol**	11.4%	40.0%	0.194	0.006*
7.	esters	diethyl phtalate	11.4%	8.6%	1.376	0.690

Groups: 1 – oral squamous cell carcinoma (OSCC) subjects; 2 – oral potentially malignant disorder (OPMD) subjects. * statistically significant; **bold** – significant VOCs.

Table 5. Distribution of volatile organic compounds (VOCs) among the oral potentially malignant disorder (OPMD) and healthy subjects (based on the *p*-value and the odds ratio (*OR*))

No.	VOC class	VOCs	Group 2	Group 3	*OR*	*p*-value
1.	organic acids	**mercaptoacetic acid**	28.6%	10.0%	3.600	0.039*
		pentanoic acid, 3-methyl-	2.9%	2.5%	1.140	0.924
		propanoic acid, 2-methyl-	62.9%	37.5%	2.820	0.028*
		benzeneacetic acid	20.0%	0.0%	0.000	0.003*
		butanoic acid, 3-methyl-	31.4%	22.5%	1.570	0.383
		2,6-dihydroxybenzoic acid	17.1%	10.0%	1.860	0.364
		heptanoic acid	14.3%	7.5%	2.050	0.342
		boric acid	2.9%	2.5%	1.140	0.924
		methanesulfonylacetic acid	5.7%	2.5%	2.360	0.479
		butanoic acid, 2-methyl-	20.0%	12.5%	1.750	0.377
		3-hydroxy-3-phenylpropionic acid	8.6%	0.0%	0.000	0.059*
		5-methylhexanoic acid	8.6%	0.0%	0.000	0.059*
		octanoic acid	2.9%	2.5%	1.140	0.924
		mandelic acid	5.7%	2.5%	2.360	0.479
		2-butenoic acid	8.6%	0.0%	0.000	0.059*
2.	alcohols	2-furanmethanol	14.3%	10.0%	2.077	0.232
		2,3-butanediol	28.6%	10%	1.500	0.569
		propylene glycol	28.6%	0.0%	0.000	0.001*
		1-dodecanol	5.7%	2.5%	2.360	0.479
3.	ketones	**acetoin**	54.3%	10.0%	10.680	<0.001*
		2-piperidinone	94.3%	87.5%	2.350	0.314
		2,5-dimethylfuran-3,4(2H,5H)-dione	25.7%	10.0%	3.110	0.073
4.	acid amides	acetamide	88.6%	70.0%	3.320	0.050*
5.	heterocyclic compounds	**indole**	14.3%	0.0%	0.000	0.013*
6.	phenols	**phenol**	40.0%	5.0%	12.66	<0.001*

Groups: 2 – oral potentially malignant disorder (OPMD) subjects; 3 – healthy controls. * statistically significant; blue – significant VOCs in the OPMD patients; **bold** – significant VOCs.

Table 6. Distribution of volatile organic compounds (VOCs) among the oral squamous cell carcinoma (OSCC) and healthy subjects (based on the *p*-value and the odds ratio (*OR*))

No.	VOC class	VOCs	Group 1	Group 3	*OR*	*p*-value
1.	organic acids	**butanoic acid**	77.10%	25.0%	10.125	<0.001*
		phosphonic acid	20.0%	12.5%	1.750	0.377
		pentanoic acid, 3-methyl-	2.9%	2.5%	1.147	0.924
		formic acid	11.4%	2.5%	5.032	0.122
		pentanoic acid, 4-methyl-	40.0%	2.5%	17.87	<0.001*
		pentanoic acid	14.3%	7.5%	2.056	0.342
2.	alcohols	**3-furanmethanol**	14.3%	0.0%	0.000	0.013*
		1,2-propanediol	8.6%	0.0%	0.000	0.059*
		1-tridecanol	0.0%	15.0%	0.000	0.017*
3.	ketones	**2-propanone, 1-hydroxy-**	48.6%	2.5%	36.830	<0.001*
		2-piperidinone	88.6%	87.5%	1.107	0.887
		2-hydroxy-gamma-butyrolactone	28.6%	2.5%	6.500	0.061
		2,5-dimethylfuran-3,4(2H,5H)-dione	28.6%	10.00%	3.600	0.039*
		1,2-cyclopentanedione	20.0%	0.0%	0.000	0.059*
4.	alkanes	**1-chloro-dodecane**	0.0%	30.0%	0.000	<0.001*
		butane, 1,4-bis(9,10-dihydro-9-methylanthracen-10-yl)-	11.4%	2.5%	5.032	0.122
5.	acid amides	propanediamide	20.0%	15.0%	1.417	0.568
		propanamide, 2-hydroxy-	8.6%	0.0%	0.000	0.059*
6.	esters	diethyl phtalate	11.4%	5.0%	1.161	0.842
7.	aldehydes	2,5-dihydroxybenzaldehyde	2.9%	2.5%	1.147	0.924

Groups: 1 – oral squamous cell carcinoma (OSCC) subjects; 3 – healthy controls. * statistically significant; red – significant VOCs in the OSCC patients; **bold** – significant VOCs.

The OPMD VOC models derived from the metabolic analysis demonstrated good separation from OSCC and healthy controls, highlighting the diagnostic potential of this non-invasive analytical approach. Eleven VOCs were found to be differentially expressed in OPMDs as compared to OSCC and controls. The categories of OPMDs among the subjects of this study included OL (both homogenous and non-homogenous), SMF and OLP. Six acids, 1 alcohol, 1 ketone, 1 acid amide, 1 heterocyclic compound, and 1 phenol were the VOCs with the levels significantly higher amongst the OPMD subjects.

Organic acids, like benzeneacetic acid, mercaptoacetic acid, 2-butenoic acid, propanoic acid, 2-methyl-, 5-methylhexanoic acid, and 3-hydroxy-3-phenyl propionic acid, were found predominantly. It is known that propionibacteria, oral commensal bacteria, convert carbohydrates to short-chain fatty acids (SCFA), especially propanoic acid, through anaerobic metabolism. Propanoic acid can also be produced during metabolism and can be generated from propionyl-CoA, an end-product in the altered metabolism of the amino acids valine, isoleucine, threonine, and methionine, as well as odd-chain fatty acid oxidation and the degradation of cholesterol.[15] A similar alteration of both propionyl-CoA and amino acid metabolism is observed in OL.[16] In view of the above, the level of propanoic acid, 2 methyl- was higher in the OPMD group as compared to controls in the present study. The results of this study are in agreement with those presented by Wei et al., who identified the amino acids valine and phenylalanine for distinguishing OL from OSCC.[17] Mercaptoacetic acid was seen in 66.7% of OL subjects. It is among substances that cause halitosis – volatile sulfur-containing compounds, such as hydrogen sulfide, methyl mercaptan and dimethyl sulfide, which in turn are associated with periodontitis. Given that more than 75% of the OPMD subjects had a history of tobacco use, this finding is justified. On the other hand, 2-butenoic acid increases glycolysis and elevates cancer cell growth through modulating the p53-dependent pathway in response to nutrition depletion, thereby making it a potential biomarker for malignant transformation. 5-methylhexanoic acid and 5-methylsalicylic acid were found in most of the OL and OLP patients; however, there are no reports indicating their role as biomarkers in OL and OLP.

Amongst alcohols, propylene glycol was found in most of the OPMD patients (28.6%). Propylene glycol is reportedly used as a humectant in tobacco products, and the majority of patients in this group had a history of tobacco use. In the ketonic group, acetoin was significantly present among OL patients. However, there is a lack of literature about these VOCs in OPMDs; the elevation of these compounds can be attributed to the tobacco use status of the

patients, as acetoin is an ingredient of tobacco products. Acid amides and heterocyclic compounds, like indole, were also observed. Although there are no previous studies on the role of acetamide as a VOC biomarker in OPMDs, an in-vitro study by Sakano et al. found that amidases, which catalyze the hydrolysis of the compounds containing an amide group, are widely distributed in mammalian organs.[18] Acetamide was hence proposed as a possible substrate for amidases to produce ultimate carcinogens. The heterocyclic VOC indole was elevated in the OPMD group, which supports a previous study by Ishikawa et al., who showed indole-3-acetate, which is the conjugate base of indole, as a significant volatile biomarker for OLP.[19]

Twelve VOCs had statistically significantly higher levels in the OSCC groups as compared to the OPMD and control group. Organic fatty acids (33.3%), ketones (33.3%), alcohols (16.6%), alkanes (8.3%), and acid amides (8.3%) were the major classes of VOCs noted in the OSCC subjects.

Lipogenesis appears to be enhanced during carcinogenesis in order to support the tumor demand for cell membrane constituents. Also, free fatty acids are important in cell signaling in neoplasms.[20] This may explain the elevated levels of acid VOCs in OSCC subjects in this study. Butanoic acid was seen to be highly significant ($p = 0.001$) among the acids. Butanoic acid is an extracellular metabolite from periodontopathic bacteria, thought to play an important role in the progression of periodontitis through its contribution to the destruction of gingival tissues and the modulation of local immunity at gingival sites.[21] In addition, it has been reported that butanoic acid promotes the migration of normal and neoplastic epithelial cells.[22] The percentage of patients with butanoic acid detected was observed to gradually increase from the healthy controls to OPMD and further to the OSCC group. Apart from butanoic acid, pentanoic acid, 4-methyl-, 3-furanmethanol, 2-propanone,1-hydroxy-, 2-hydroxy-gamma-butyrolactone, and 1,2-cyclopentanedione also followed a similar trend, giving these VOCs strong potential to serve as early biomarkers in OSCC. Formic acid (11.4%) is thought to be a breakdown product of formaldehyde, which is also a component of tobacco smoke. Considering that 4 out of 10 active tobacco users in the OSCC group were smokers, it might be the reason for its occurrence in addition to its formation in cancer metabolism.

2-propanone, 1-hydroxy- was found to be significantly elevated amongst the group of ketones ($p = 0.001$) in comparison with both OPMD and healthy controls, which is in accordance with the study by Shigeyama et al., who dealt with the identification of salivary VOCs in oral cancer.[23] Ketones may function as chemoattractants and stimulate the migration of epithelial cancer cells, promoting primary tumor growth.[24] Ketone production is also linked to greater fatty acid oxidation rates, which have been reported in numerous malignancies. Cancer cells also exhibit altered glucose metabolism known as the Warburg effect, in which the production of their energy shifts from the Krebs cycle to glycolysis, which could explain the appearance of ketonic VOCs in this study. However, as the concentration of ketones in human fluids or breath fluctuates with certain activities, such as fasting, exercising and eating, some experts advise against using ketones as biomarkers.[25] Alcohols were also found in most of the OSCC patients, possibly due to the action of cytochrome P450 enzymes, which hydroxylate the lipid peroxidation biomarkers to produce alcohols. Phillips et al. published an article in 1999 identifying 22 VOCs in the exhaled breath of patients with lung cancer, 15 of which were alkanes.[26] Similarly, we found increased butane, 1,4-bis(9,10-dihydro-9-methylanthracen-10-yl)- levels, an alkane to be statistically significant ($p = 0.053$). The origin of this compound is related to oxidative stress; it is mainly formed during the lipid peroxidation of PUFA constituents of biological membranes, leading to the degradation of phospholipids and, eventually, cellular deterioration.[27] It is thought by some researchers to be a secondary product of oxidative stress[27]; however, others disagree with this hypothesis.[28] In addition, acid amides were also found to be significantly high ($p = 0.037$) in the OSCC group, thereby representing a strong biomarker for OSCC.

When comparing the VOC profiles between the OSCC and OPMD patients, it was the revealed that the salivary metabolites had the potential to discriminate OSCC from OPMD patients. Among the 14 identified metabolites, 6 compounds displayed statistically significant differences between both groups ($p \leq 0.05$) (Table 4).

The observed chemical classes of VOCs were acids, ketones and alkanes. Phosphonic acid, formic acid, 2-propanone, 1-hydroxy-, and butane, 1,4-bis(9,10-dihydro-9-methylanthracen-10-yl)- were the differentially expressed VOCs in OSCC in comparison with OPMDs. Except for mercaptoacetic acid and phenol, all metabolites were decreased in OPMDs as compared to OSCC. These up- and downregulations of metabolites may be due to the involvement of metabolites in different metabolic pathways simultaneously.[29] Periodontitis is also independently associated with OPMDs.[30] This link between periodontitis and OPMDs may explain the occurrence of mercaptoacetic acid and phenol in the OPMD patients, and their strong distinction as a volatile biomarker from OSCC.

Ten VOCs were found to be statistically significant in the comparison between OSCC and healthy controls (Table 6). There were 2 acids, 3 alcohols, 3 ketones, and 2 alkanes found. In addition to butanoic acid, pentanoic acid, 4-methyl- was the most significant VOC noted ($p > 0.001$) in OSCC patients. The findings of the present study are consistent with the previous research reporting elevated levels of branched-chain fatty acids in cancer-related volatilomic profiles.[31] The levels of 2 alcohols were found to be higher in the OSCC group, which could

be attributed to the cytochrome P450 enzymatic action, while 1 alcohol – 1-tridecanol, which is a flavoring and fragrance agent, was observed in only 15% of healthy controls in comparison with OSCC patients (0%), and hence was eliminated as a biomarker for OSCC. In addition to this, 1-chloro-dodecane, which is an alkane, was also eliminated as a biomarker for OSCC, as it was present in only 30% of healthy controls and completely absent in OSCC patients.

The remaining VOCs illustrated a rise amongst the OSCC group of patients, although they depicted relatively low statistical significance. Although not statistically significant, 2,5-dihydroxybenzaldehyde ($OR > 1$) was also observed in 2.9% of OSCC subjects. Aldehydes are generated as intermediates in cytotoxic processes during signal transduction, genetic regulation or cellular proliferation through alcohol metabolism, the reduction of hydroperoxide by cytochrome P450 as a secondary product of lipid peroxidation and the detoxification processes related to smoking. In the particular case of cancer, previous studies reported an increment in the activity of aldehyde dehydrogenases (ALDHs), with cancer cell propagation resulting in the growth and proliferation of tumor cells, thereby implying an increase in the concentration of aldehydes in blood and saliva. Increased aldehyde production in cancer patients may be due to changes in membrane lipid composition and increased oxidative stress in tumor cells. Furthermore, increased levels of certain unsaturated fatty acids in the membranes of tumor cells may increase the production of certain aldehydes through lipid peroxidation. Aldehyde dehydrogenases and alcohol dehydrogenases (ADHs) are two abundant enzyme groups in the human liver. Aldehydes can be irreversibly oxidized to carboxylic acids by ALDHs or reduced to their corresponding alcohols by ADHs, thereby establishing a relation with organic acids and alcohols in accordance with the VOCs obtained in this study.

The analysis of the VOC profiles based on the pTNM staging showed that 12 VOCs were statistically significant ($p \leq 0.05$) (Fig. 10). The predominant classes of VOCs were organic acids, ketones, acid amides, ethers, and furans. Oxalic acid (25%), 4-hydroxybutyric acid hydrazide (25%), boronic acid (25%), and tridecanoic acid (25%) were the significantly elevated acids found during the early stages, and the ethers diisoamyl ether (25%) and mephenesin (25%) were elevated as well. In addition, ketones like dihydroxyacetone (25%), acid amides like isobutyramide (25%) and furanal derivates like 5-(hydroxymethyl)furfural (25%) were also observed to be distinctively higher in the early stages in comparison with the advanced stages. Diethyl phthalate and 2-hydroxy-gamma-butyrolactone were significantly higher in all subjects with stage-II cancer (100%), providing a potential early biomarker. In addition to these, the acid amide propanamide, 2-hydroxy- (50%) was also observed equally often in both stage I and stage II. Diethyl phthalate and 2-hydroxy-gamma-butyrolactone were the only VOCs present in the advanced stages, i.e. stage III and stage IV. However, there have been no literature reports concerning the salivary VOCs based on the pTNM staging of OSCC till date, although similar studies have been done in breath volatilomics. Fu et al. found the exhaled breath concentration of 2-butanone significantly higher in patients with stage-I lung cancer in comparison with the advanced stages.[32] In contrast, Oguma et al. reported increased concentrations of cyclohexane and xylene in advanced lung cancer,[33] which does not agree with the results of the present. In another study, Corradi et al. showed that although lung cancer patients had higher levels of ethylbenzene in their breath, the difference between early-stage lung cancer patients and control subjects was less pronounced.[34] Peled et al. analyzed breath samples using GC–MS; however, the analysis did not reveal any significant differences between early-stage and late-stage lung cancer, nor did it show any distinction among the sub-histological types.[35]

As the prognosis and treatment options are critically dependent on the histology of the cancer, the salivary volatilomic profile in the OSCC patients was also identified based on the histopathological staging. The obtained metabolomic profile is in agreement with the literature, which supports the correlation between the pTNM and histopathological staging. Ten VOCs were statistically significant ($p \leq 0.05$) (Fig. 11). The main classes of VOCs were organic acids, ketones, acid amides, ethers, and furans. Oxalic acid (25%), boronic acid (25%) and tridecanoic acid (25%) were significant in the early histological stages. In addition, ketones like dihydroxyacetone (25%) and 1,2-cyclopentanedione (45%), acid amides like isobutyramide (25%), and furanal derivates like 5-(hydroxymethyl)furfural (25%) were also observed to be distinctively higher in the early stages in comparison with the advanced stages. Diethyl phthalate and 2-hydroxy-gamma-butyrolactone were the only VOCs present in the advanced stage, i.e., in poorly differentiated OSCC, corresponding to the pTNM stages III and IV. These VOC profiles can potentially serve as non-invasive prognostic biomarkers in OSCC. However, there is a dearth of studies in the literature related to the identification of salivary VOCs based on the histopathological staging of OSCC. Few other studies showed that the histopathological staging had no significant impact on VOCs.[34,35]

Conclusions

The present study proposes a salivary VOC-based metabolomic profiling approach for patients with OSCC and OPMDs, compared with healthy controls, to support the discovery of clinically relevant biomarkers with potential diagnostic, prognostic and therapeutic applications. The findings provide volatilomic insights into the salivary metabolite alterations associated with OSCC and

OPMDs. Although similar classes of compounds were detected in both disease groups, individual VOC profiles showed distinct variations. Additionally, the VOC profiles demonstrated significant associations with gender, age, the type of tobacco use, the pTNM classification, and the histopathological staging. These VOCs may represent tumor-specific candidate biomarkers suitable for screening OSCC and OPMDs. However, larger-scale studies are required to validate these findings and establish standardized protocols for their clinical implementation.

Limitations and recommendations

Challenges include the strong influence of confounding factors, such as food consumption, medications, physical activity, comorbid non-cancer diseases, and the normal gut microbiota, all of which can alter the pattern of generated VOCs. Care must therefore be taken to minimize the impact of these factors. Another limitation is that the origin and biological sources of many VOCs remain unclear, necessitating further research to better understand the altered metabolic pathways associated with their production.

The study population was small; therefore, the findings should be considered preliminary, and future studies should include larger sample sizes. Identifying distinguishable VOC fingerprints or the chemical groups associated with different cancers may facilitate early detection, provide insights into the mechanisms underlying cancer development and progression, and ultimately enable the manipulation of the altered metabolic pathways. Furthermore, the development of a portable, sensor-based point-of-care device for VOC detection would be invaluable in clinical practice for early diagnosis, monitoring disease states and assessing post-therapy outcomes.

Ethics approval and consent to participate

Ethical clearance was obtained from the Ethics Committee for Human Trials of M.S. Ramaiah University of Applied Sciences, Bengaluru, India (No. EC-2020/PG/36). Informed written consent was obtained from patients and controls after explaining the nature of the study.

Data availability

The patient datasets used in the current study are confidential and cannot be shared.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Effect of colchicine administration on interleukin-1β and nitric oxide expression at the early stage of atherosclerosis in atherosclerosis Wistar rat model

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Abstract

Background. The basic mechanisms underlying early atherosclerosis remain controversial. Several theories centered on lipid accumulation have been proposed, but increasing evidence highlights the central roles of inflammation and endothelial dysfunction in the initiation of the disease. Two major processes – chronic lipid-driven injury and maladaptive inflammatory and cellular responses – are closely involved in early atherogenesis and offer potential targets for new management strategies in atherosclerotic cardiovascular disease (ASCVD).

Objectives. The aim of the present study was to evaluate the effects of colchicine compared with atorvastatin on the expression of interleukin-1β (IL-1β), a key pro-inflammatory cytokine, and nitric oxide (NO), a protective mediator, both of which play important roles at the early stages of atherosclerosis.

Material and methods. This was an in vivo experimental study. A total of 20 male Wistar rats (*Rattus novergicus*) were divided into 4 groups: the control (normal) group (N); the dyslipidemia group fed an atherogenic diet (DL); the dyslipidemia group receiving both an atherogenic diet and colchicine (DLK); and the dyslipidemia group receiving both an atherogenic diet and atorvastatin (DLA). All kinds of treatment were administered for 14 days.

Results. The results showed that colchicine and atorvastatin were equally effective in terms of IL-1β reduction ($p > 0.05$). Yet, the data also showed that the NO levels were significantly higher in the DLK group as compared to the DLA group ($p < 0.05$).

Conclusions. In the early development of atherosclerosis, colchicine was significantly more effective than atorvastatin in increasing the NO levels and demonstrated a comparable ability to reduce the IL-1β levels. These findings suggest that colchicine may offer superior benefits as a primary preventive therapy in populations at risk for ASCVD.

Keywords: atherosclerosis, nitric oxide, interleukin-1β, colchicine

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Highlights

- Colchicine shows superior effectiveness in increasing the NO levels as compared to atorvastatin, offering potential benefits for early atherosclerosis prevention.
- Colchicine demonstrates comparable ability to reduce the IL-1β levels, suggesting its potential as a primary preventive therapy for populations at risk of atherosclerotic cardiovascular disease (ASCVD).

Introduction

Cardiovascular disease (CVD) remains a major global health threat and is the leading cause of death worldwide. Among its forms, atherosclerotic cardiovascular disease (ASCVD) continues to account for the largest share of cases, contributing substantially to global morbidity and mortality.[1] The basic mechanisms underlying early atherosclerosis remain controversial.[2] Statins have long been the cornerstone of atherosclerosis prevention and treatment. In addition to their lipid-lowering action, statins exert pleiotropic (cholesterol-independent) effects that contribute to plaque regression. They also help stabilize atheromatous lesions by increasing fibrous cap thickness and enhancing macrocalcification.[3,4] Emerging theories highlight the central roles of inflammation and endothelial dysfunction in the development of atherosclerosis, offering new insights and potential strategies for the management of ASCVD.[2,5] Nitric oxide (NO) functions as a vasodilator and possesses antiplatelet, antiproliferative, anti-inflammatory, and antioxidant properties. Reduced NO bioavailability leads to endothelial dysfunction, a key early causal factor in the development of atherosclerosis.[6] Inflammation plays a crucial role in atherogenesis through the activation and proliferation of macrophages, endothelial cells and vascular smooth muscle cells. Macrophage-driven inflammatory responses involve the release of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interleukin-12 (IL-12), and they serve as a major source of reactive oxygen species (ROS) within atherosclerotic lesions.[7,8] During the progression of atherosclerosis, IL-1β plays a dominant role in inducing endothelial dysfunction, and activating leukocytes and proteases. At the complication stage, IL-1β contributes to platelet activation, which can trigger the rupture of atheromatous plaques, and subsequently lead to thrombosis.[9,10]

Numerous studies have investigated the effects of anti-inflammatory drugs on ASCVD and their association with major adverse cardiac events (MACE), including colchicine. Colchicine exerts its therapeutic effects by targeting multiple stages of the inflammatory process. Unlike other anti-inflammatory drugs or glucocorticoids, colchicine operates independently of the arachidonic acid pathway. It inhibits neutrophil adhesion to the vascular endothelium, increases the leukocyte cyclic adenosine monophosphate (cAMP) levels, suppresses IL-1 production by activated neutrophils, and blocks TNF-α receptors in macrophages and endothelial cells.[11–13] However, the potential benefits of colchicine, particularly its effects at the molecular level during the early phase of atherosclerosis and its role as a primary preventive therapy in populations at risk for ASCVD, still require further investigation.

The present study aimed to evaluate the effects of colchicine on the concentration of the inflammatory marker IL-1β, which plays a key role at the early stages of atherosclerosis, and on NO, a vasodilator that protects endothelial cells. These effects were compared with those of atorvastatin, a well-established therapeutic agent.

Material and methods

This experimental study involved twenty 4-week-old male Wistar rats (*Rattus norvegicus*), weighing 150–200 g, obtained from Bogor Agricultural University (IPB), Indonesia. The rats were housed in sterile stainless steel cages in a temperature-controlled environment (23°C) with a 12-hour light/dark cycle. They were kept in a well-ventilated area with ad libitum access to tap water and a standard pellet diet. The rats were randomly assigned to 4 groups, each consisting of 5 animals: the control (normal) group (N); the dyslipidemia group fed an atherogenic diet (DL); the dyslipidemia group receiving both an atherogenic diet and colchicine (DLK); and the dyslipidemia group receiving both an atherogenic diet and atorvastatin (DLA).

After a 2-week acclimation period, 5 rats were fed a normal diet, while 15 rats were given an atherogenic diet ad libitum for 8 weeks. The atherogenic diet consisted of vitamin D3, 0.2% cholic acid, 2% egg yolk, 5% goat fat, and 92.8% corn rice. Following the 8-week feeding period, the low-density lipoprotein (LDL) levels were assessed to evaluate the effects of atherosclerosis induction. A previous study showed that an 8-week atherogenic diet significantly increased the LDL levels and induced foam cell formation in male Sprague–Dawley albino rats, thereby impacting their lipid profile.[14]

The treatment phase began in week 9, when the rats were 15 weeks old. During this phase, the animals were fed according to the study design described above. The rats in the treatment groups received therapeutic doses of colchicine (0.5 mg/day) or atorvastatin (40 mg/day) for

14 days. At the end of the treatment period, euthanasia was performed by the researchers and the laboratory staff through the intraperitoneal administration of ketamine (Ilium Ketamil; Troy Laboratories, Sydney, Australia) and xylazine (Xyla; Interchemie, Venray, the Netherlands). Blood samples were collected directly from the heart and transferred into Venoject® tubes. Plasma was separated by centrifugation, using a microcentrifuge (MC-12; Benchmark Scientific Inc., Sayreville, USA) at 3,000 rpm for 10 min, and then immediately stored at –80°C. The plasma samples were later used to measure the IL-1β and NO concentrations.

Measurement of the IL-1β and NO concentrations

The measurement of the IL-1β and NO concentrations in the rat plasma samples was performed using the enzyme-linked immunosorbent assay (ELISA) method. The IL-1β levels were quantified using the Rat IL-1β ELISA Kit (cat. No. E-EL-R0011; Elabscience®, Wuhan, China), and the NO concentrations were measured using the Rat NO ELISA Kit (cat. No. E-BC-K035-M; Elabscience).

The competitive ELISA procedure began with coating the wells with the antigen. A total of 100 µL of the standard and the test sample was added to each well, except for the blank. The plates were incubated at 37°C for 1 h, followed by the addition of 50 µL of substrate A and 50 µL of substrate B to each well. The plates were then incubated for 10–15 min at 37°C, protected from light. The reaction was terminated by adding 50 µL of a stop solution to each well. After 5 min, absorbance was measured at 450 nm, using a microplate reader (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad, Hercules, USA).

Statistical analysis

Data normality and the homogeneity of variances were assessed using the Shapiro–Wilk test ($p > 0.05$) and Levene's test, respectively. The one-way analysis of variance (ANOVA) was used to evaluate the effects of colchicine and atorvastatin administration on the IL-1β and NO concentrations. Post-hoc analysis was subsequently performed to determine pairwise differences among the groups. Statistical analysis was conducted using IBM SPSS Statistics for Windows, v. 20.0 (IBM Corp., Armonk, USA).

Results

Atherosclerosis was induced by administering an atherogenic diet for 8 weeks. The Shapiro–Wilk test confirmed that the data was normally distributed ($p > 0.05$). As shown in Table 1, the mean LDL levels after atherosclerosis induction in the DL, DLK and DLA groups were 72.3 ±8.9 mg/dL, 74.0 ±10.6 mg/dL and 73.8 ±9.9 mg/dL, respectively. In contrast, the normal group had a mean LDL level of 23.8 ±5.3 mg/dL.

Effect of colchicine on the LDL levels

The study also evaluated the effect of colchicine on reducing the LDL levels. The results demonstrated that administering 0.5 mg of colchicine for 14 days effectively lowered the LDL levels in rats with atherosclerosis. The mean reduction in the LDL levels in the DLK group was 32.8 ±6.2 mg/dL ($p < 0.05$), as presented in Table 2.

Effect of colchicine on the IL-1β levels

The results showed that the mean IL-1β levels were 56.5 ±19.6 µ/mmol in the DL group, 32.4 ±5.7 µ/mmol in the DLK group and 39.7 ±11.5 µ/mmol in the DLA group ($p < 0.05$), as presented in Table 3. These findings indicate a significant difference in the IL-1β levels among the treatment groups. In contrast, IL-1β was not detected in the N group.

Table 1. Mean low-density lipoprotein (LDL) levels after atherosclerosis induction

Group	LDL level [mg/dL]
N	23.8 ±5.3
DL	72.3 ±8.9
DLK	74.0 ±10.6
DLA	73.8 ±9.9

Data presented as mean ± standard deviation (*M ±SD*).
Groups: N – control (normal) group; DL – dyslipidemia group with an atherogenic diet; DLK – dyslipidemia group (atherogenic diet + colchicine); DLA – dyslipidemia group (atherogenic diet + and atorvastatin).

Table 2. Comparison of the low-density lipoprotein (LDL) levels before and after colchicine administration in the DLK group

Colchicine administration in DLK	LDL level [mg/dL]	Mean difference [mg/dL]	95% *CI*	*p*-value
Before	74.0 ±10.6	32.8 ±6.2	25.0–40.5	<0.000*
After	41.2 ±4.4			

Data presented as *M ±SD*.
DLK – dyslipidemia group (atherogenic diet + colchicine); *CI* – confidence interval. * statistically significant.

Table 3. Mean interleukin-1β (IL-1β) levels for each treatment group

Group	Number of samples	IL-1β level [µ/mmol]	*p*-value
DL	5	56.5 ±19.6	0.004*
DLK	5	32.4 ±5.7	
DLA	5	39.7 ±11.5	

Data presented as *M ±SD*.
Groups: DL – dyslipidemia group with an atherogenic diet; DLK – dyslipidemia group (atherogenic diet + colchicine); DLA – dyslipidemia group (atherogenic diet + and atorvastatin). * statistically significant.

Post-hoc analysis was conducted to identify which group had the lowest IL-1β levels. As shown in Table 4, there was no significant difference in the mean IL-1β levels between the DLK and DLA groups ($p > 0.05$). However, a significant difference of 24.1 µ/mmol was observed between the DL and DLK groups ($p < 0.05$). In contrast, no significant difference was found between the DL and DLA groups ($p > 0.05$).

Effect of colchicine on the NO levels

The results (Table 5) showed that the mean NO levels were 216.04 ±20.39 µ/mmol in the DLK group and 141.44 ±18.05 µ/mmol in the DLA group. Meanwhile, the NO levels in the N and DL groups were 263.00 ±16.18 µ/mmol and 107.44 ±8.71 µ/mmol, respectively ($p < 0.05$). These findings indicate a significant difference in the NO levels among the groups. As expected, the N group, serving as the negative control, exhibited relatively higher NO levels, with a mean value of 250.55 ± 8.05 µ/mmol.

Table 4. Post-hoc analysis of the interleukin-1β (IL-1β) levels among the treatment groups

Pairwise comparisons	Mean difference [µ/mmol]	95% *CI*		*p*-value
		min	max	
DL vs. DLK	24.1	0.35	47.99	0.046*
DL vs. DLA	16.8	6.99	40.65	0.219
DLK vs. DLA	7.3	16.49	31.15	1.000

Groups: DL – dyslipidemia group with an atherogenic diet; DLK – dyslipidemia group (atherogenic diet + colchicine); DLA – dyslipidemia group (atherogenic diet + and atorvastatin). min – minimum; max – maximum; * statistically significant.

Table 5. Mean nitric oxide (NO) levels for each treatment group

Group	Number of samples	NO level [µ/mmol]	*p*-value
DL	5	107.44 ±8.71	
DLK	5	216.04 ±20.39	0.000*
DLA	5	141.44 ±18.05	

Data presented as *M* ±*SD*.
Groups: DL – dyslipidemia group with an atherogenic diet; DLK – dyslipidemia group (atherogenic diet + colchicine); DLA – dyslipidemia group (atherogenic diet + and atorvastatin). * statistically significant.

Table 6. Post-hoc analysis of the nitric oxide (NO) levels among the treatment groups

Pairwise comparisons	Mean difference [µ/mmol]	95% *CI*		*p*-value
		min	max	
DL vs. DLK	108.60	79.59	137.62	0.000*
DL vs. DLA	34.00	4.98	63.02	0.021*
DLK vs. DLA	74.60	45.58	103.62	0.000*

Groups: DL – dyslipidemia group with an atherogenic diet; DLK – dyslipidemia group (atherogenic diet + colchicine); DLA – dyslipidemia group (atherogenic diet + and atorvastatin). * statistically significant.

Post hoc analysis revealed a significant difference in the NO levels of 74.60 µ/mmol between the DLK and DLA groups ($p < 0.05$). Similarly, a significant difference of 108.60 µ/mmol was observed between the DLK and DL groups ($p < 0.05$). Additionally, the mean NO level in the DLA group was significantly higher than in the DL group, with a difference of 34.00 µ/mmol ($p < 0.05$) (Table 6).

Discussion

Normally, male Wistar rats (*Rattus norvegicus*) have the LDL levels ranging from 10 to 54 mg/dL.[15] An increase in the LDL fraction in plasma, as observed in the atherogenic model group, leads to dyslipidemia. Elevated LDL levels are a major risk factor for atherosclerosis, as they promote the accumulation of lipoproteins in the intimal layer, stimulate macrophage and monocyte adhesion, and trigger the migration of sub-endothelial smooth muscle cells, thereby accelerating the formation of atheromatous plaques.[16,17] A previous study showed similar results, demonstrating that an 8-week high-fat diet significantly increased the triglyceride and LDL levels, decreased the high-density lipoprotein (HDL) levels, and induced the formation of aortic atheromatous plaques in male Sprague–Dawley albino rats.[14]

The mean decrease in the LDL levels in the DLK group was 32.8 ±6.2 mg/dL ($p < 0.05$) after administering 0.5 mg of colchicine for 14 days, indicating that colchicine effectively lowers the LDL levels. The precise mechanism underlying this effect remains unclear. Notably, the consistent reduction in LDL across all DLK group samples is an interesting finding, suggesting that colchicine plays a significant protective role against the endothelial cell damage caused by cholesterol crystals. Colchicine has been shown to reduce the formation of cholesterol crystal-induced ROS, thereby inhibiting the activation of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome and the associated inflammatory response, ultimately ameliorating endothelial cell pyroptosis. These effects highlight the potential of colchicine as a promising therapeutic agent for the prevention and treatment of atherosclerosis.[18]

This finding is consistent with a previous study conducted on 24 Sprague–Dawley rats used as a model of atherosclerosis with a high-fat diet.[19] In that study, the administration of 0.5 mg colchicine for 5 weeks resulted in decreased triglyceride and LDL levels, along with a significant increase in the HDL levels.[19]

Data analysis showed no significant difference in the mean IL-1β levels between the DLA and DLK groups ($p > 0.05$). Based on these results, it can be concluded that colchicine and atorvastatin exhibited similar efficacy in suppressing IL-1β expression during the early stages of atherosclerosis in this study.

Colchicine inhibits the activation of NLRP3 inflammasomes in macrophages in response to stimuli such as cholesterol crystals and ROS, leading to reduced production of IL-1β and other pro-inflammatory cytokines, including TNF-α and IL-6, in atherosclerotic lesions, thereby preventing disease progression.[20,21] Similarly, atorvastatin suppresses IL-1β expression by inhibiting NLRP3 inflammasome activity through phagolysosomal pathways.[22] This shared mechanism explains the comparable effectiveness of colchicine and atorvastatin in suppressing IL-1β expression, as observed in this study.

Immunofluorescence staining has demonstrated that the assembly of the NLRP3 inflammasome into its active complex requires microtubule-mediated transport. Colchicine inhibits microtubule polymerization and promotes microtubule degradation, thereby effectively suppressing the inflammatory response.[23,24]

This study supports previous findings on the effectiveness of colchicine in reducing the monocyte IL-1 levels in patients with acute coronary syndrome (ACS) by lowering the protein levels of pro-caspase-1 and caspase-1.[11] Caspase-1 plays a key role in the NLRP3-mediated inflammatory response, including the activation of IL-1β and IL-6. It cleaves pro-IL-1 and pro-IL-18 into their active forms, so the inhibition of caspase-1 naturally reduces the downstream levels of active IL-1.[24]

The highest mean NO level was observed in the DLK group, at 216.04 ±20.39 μ/mmol. These results suggest that colchicine is more effective than atorvastatin in increasing the NO levels, a key mediator of vasodilation that protects endothelial cells during the early stages of atherosclerosis.

Colchicine significantly increases the expression of phosphorylated AMP-activated protein kinase (AMPK), a critical regulator of energy metabolism. In the prevention and treatment of atherosclerosis, AMPK promotes cholesterol excretion, enhances fatty acid oxidation and inhibits inflammatory processes.[18] The activation of the AMPK pathway also modulates vascular endothelial function, suppresses ROS production and reduces oxidative stress during the early stages of atherosclerosis. This mechanism helps explain the observed increase in the NO levels, a vasodilator that protects endothelial cells in the early development of atherosclerosis in this study.[18,25]

A similar study reported comparable results, showing that a single therapy with colchicine administered to hyperlipidemic rats at the early stages of atherosclerosis improved both inflammation and endothelial function, even independently of the lipid-lowering effects.[22]

An in vitro study reported similar findings, showing that colchicine reduced ROS formation and increased the NO levels, thereby alleviating oxidative stress.[26] These effects create a favorable environment for preventing the formation and progression of atherosclerotic lesions. In other words, colchicine exerts beneficial effects in both the primary and secondary prevention of coronary heart disease (CHD).

Conclusions

In the early development of atherosclerosis, colchicine was significantly more effective than atorvastatin in increasing the NO levels and demonstrated a comparable ability to reduce the IL-1β levels. These findings suggest that colchicine may offer superior benefits as a primary preventive therapy in populations at risk for ASCVD.

Limitations

This study focused solely on the effects of colchicine on the IL-1β and NO levels during the early development of atherosclerosis, without conducting histopathological examinations to assess atherosclerotic lesion formation and progression. Further studies are warranted to evaluate the relationship between colchicine administration, the functions of other organs and potential adverse effects.

Ethics approval and consent to participate

The study was evaluated and approved by the Research Ethics Committee at the Faculty of Veterinary Medicine of the Syiah Kuala University, Banda Aceh, Indonesia (132/KEPH/V/2021).

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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Original paper

Effect of the scanner type on the marginal gap and internal fit of two monolithic CAD/CAM esthetic crown materials: An in vitro study

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Abstract

Background. The durability of indirect restorations is significantly influenced by marginal adaptation and internal fit. The use of computer-aided design/computer-aided manufacturing (CAD/CAM) with digital impressions has reduced dental prosthesis fabrication errors, improving the long-term survivability of the restorations.

Objectives. The present study assessed the impact of intraoral and extraoral scanning methods on the marginal adaptation and internal fit of 2 different types of monolithic crowns manufactured using CAD/CAM.

Material and methods. A total of 40 three-dimensional (3D) resin-printed dies were randomly assigned to 2 groups based on the type of crown material ($n = 20$ per group). Each group was divided into 2 subgroups ($n = 10$ per group) according to the die-scanning technique: subgroup A, scanned using the intraoral scanner (IOS) Primescan; and subgroup B, scanned using the extraoral scanner (EOS) inEos X5. The digitized photos were converted into a 3D virtual crown design using CAD software. The internal discrepancy values, and the marginal gap between the 3D resin-printed die and the crown were assessed using a ×50 digital microscope. The data was checked for normality with the Kolmogorov–Smirnov test, and the Mann–Whitney *U* test was used to compare the tested groups. The collected data was analyzed at a significance level set at $p < 0.05$.

Results. The different scanning techniques used had a statistically significant effect on the vertical marginal gap and the internal fit [µm] ($p < 0.05$). As far as the crown materials are concerned, BRILLIANT Crios showed a significantly higher marginal gap as compared to Tetric CAD when scanned with inEos X5 ($p = 0.004$), whereas the differences were insignificant with regard to the internal fit ($p > 0.05$). The crown parameters tested with both scanning systems were within the clinically acceptable ranges.

Conclusions. Scanning methods and crown materials had an impact on the internal fit and vertical marginal gap of monolithic crowns.

Keywords: marginal gap, internal fit, monolithic crowns, digital scanning

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Introduction

Since its advent in 1985, the computer-aided design/computer-aided manufacturing (CAD/CAM) technology has brought significant progress in dentistry. What mostly benefited from the CAD/CAM technology is chair-side dental treatment, which involves preparing the teeth and applying restorations at a single clinical appointment.[1]

Due to advances in software and technology, numerous companies were able to develop highly precise scanners capable of capturing three-dimensional (3D) virtual images of the prepared teeth. These scanners started to be widely used in clinical dentistry to create digital models without the need for traditional impressions. Additionally, CAD software is utilized to build prostheses based on the collected data, which acts as a virtual wax-up. Owing to digital scanning techniques, the marginal accuracy of restorations has improved. The CAD/CAM technology helps to overcome some of the limitations associated with traditional impression processes by allowing quick and accurate saving of the scanned pictures without distortion.[2,3]

Digital impression systems utilize intraoral (direct digitalization) or extraoral (indirect digitalization) scanners.[4] Intraoral scanners enable direct scanning of the implant body, oral tissues and dental arches, eliminating the need for taking traditional impressions, and thus reducing patient discomfort – pain, a gag reflex and a bad taste. Intraoral scanning also enables instantaneous communication with the laboratory and the real-time assessment of the preparation. However, the quality of intraoral scanning may be adversely affected by blood, saliva and other moisture contamination, the movement of the patient or dentist, limited space within the oral cavity, and a smaller measuring area. Extraoral scanning involves scanning the impression of the dental arch or the stone model. The drawbacks of this method include the deformation of the impression material, dimensional changes in the impression material and discomfort for the patient in the event that a new impression is required.[5]

During intraoral scanning, images are acquired using a step-by-step approach. When capturing extra scans of complex angled surfaces, different angles are utilized as compared to the flat axial surface. Moreover, when scanning extensive and intricate angled areas, multiple single images are combined. The software uses the first image obtained with the scanner as a reference point, onto which subsequent images are merged. Each overlapping area introduces an error, which increases with each stitching process.[6] However, the extraoral scanner consistently captures laser plane projections and records their reflections simultaneously from all angles. Consequently, neither the area nor the complexity of the surface affects the performance of the extraoral scanner. Conversely, intraoral scanners produce fewer deviations for shorter directly measured distances and more deviations for longer directly measured distances.[7]

In comparison with veneered crowns, monolithic crowns are related to lower manufacturing costs, require less production time and preserve more tooth tissue due to the reduced ceramic thickness required. However, they also have significant drawbacks, such as increased brittleness, limited esthetics and being difficult to repair.[8]

Since many of the materials used in CAD/CAM resin composites are relatively new,[9] the data on their marginal adaptability and internal fit is still lacking. However, CAD/CAM resin blocks offer certain advantages over glass-ceramic blocks. In addition to having fewer microcracks during manufacture and less wear to the opposing dentition, they are also less fragile, which can improve the marginal adaptation of restorations.[10] This is the outcome of an industrial process that is standardized, and involves curing the material at high temperature and/or pressure values to enhance the material characteristics and maximize polymer cross-linking.[10]

Prosthetic crown success requires satisfactory marginal adaptation. The marginal gap is the distance between the edge of the prepared tooth and the cervical margin of the restoration. The presence of marginal holes in the cement increases the likelihood of disintegration, biofilm build-up, secondary caries, pulp inflammation, and periodontal disease. The recommended threshold for CAD/CAM crowns is between 50 and 100 μm, with a clinically acceptable marginal difference of less than 120 μm.[11] Marginal adaptation may be influenced by various factors, including the design of the preparation, the placement of the margin, the waxing processes, the precision of the milling system, the size of the milling bur, the thickness of the cement space, and the restorative material.[11] Additionally, the internal fit of a ceramic crown is a critical factor. The internal fit is the gap between the crown and the occlusal/incisal and axial surfaces.[12] Inadequate internal fit can decrease the fracture resistance of the restoration.[13] One of the most important contributing elements with regard to the development of CAD/CAM systems was the advancement of high-precision restorations. Recent studies have shown that CAD/CAM restorations typically exhibit small discrepancies within clinically acceptable limits, and the accuracy of the new technology matches or excels that of traditional lost-wax techniques.[14]

A limited amount of research has focused on the impact of different types of scanners on the internal fit and marginal adaptability of CAD/CAM-made crowns.[15] We aimed to assess the effects of intraoral and extraoral scanning methods on the internal fit and vertical marginal gap distance of 2 types of milled monolithic crowns. The null hypothesis stated that there would be no significant difference between the Primescan (intraoral) and inEos X5 (extraoral) scanners in terms of marginal and internal fit.

Material and methods

Die preparation

The study employed a typodont (Nissin Dental Products Inc., Kyoto, Japan) for an upper first premolar. The unprepared typodont was scanned using CEREC Primescan AC (Dentsply Sirona, Bensheim, Germany). A standardized virtual all-ceramic preparation was then performed using the Blenderfordental® CAD software, v. 3.6 (B4D, Gold Coast, Australia). The virtual preparation comprised rounded line angles, a 1.5-millimeter axial surface reduction, a 1.5-millimeter occlusal reduction, a 6-degree axial inclination, and a well-defined 1-millimeter-deep circumferential chamfer. Subsequently, the virtual preparation was 3D printed into 40 individual resin dies, using a resin 3D printer (Halot-Mage Pro; Creality, Shenzen, China) and resin material (ProShape Egypt, Cairo, Egypt).

Based on the data extracted from a study by Jalali et al.,[15] a minimum sample size of 12 (n = 6 in each group) would result in 95% power at the significance level. The sample size was increased to 10 in each group to ensure the reliability of the statistical analysis. The 40 samples were divided into 2 groups based on the type of crown material (n = 20 per group): group 1 specimens were milled from Tetric® CAD (Ivoclar Vivadent, Schaan, Liechtenstein); and group 2 specimens were milled from BRILLIANT Crios (Coltène/Whaledent, Altstätten, Switzerland) (Table 1). Subsequently, each group was divided into 2 subgroups (n = 10 per group) based on the digital scanning technique used – for subgroup A, an intraoral digital scanner (IOS) (Primescan; Dentsply Sirona) was used, while in subgroup B, an extraoral scanner (EOS) (inEos X5; Dentsply Sirona) was used.

Die scanning

Twenty dies were digitally scanned using IOS (Primescan) that required no powder, and the remaining 20 dies were digitally scanned using the laboratory EOS (inEos X5). The scanner was held quite closely over the resin die. Scanning started at the occlusal surface, proceeded to the lingual surface and ended at the buccal surface. Then, the image was automatically taken.

The scanners were calibrated before scanning. The uniform scanning procedure was followed based on the manufacturer's instructions. The same skilled operator carried out all scanning to increase repeatability and prevent any inconsistencies.

Fabrication of crowns

After determining the finish line, the dies scanned with IOS were used to create 3D virtual crown designs, utilizing the CAD software library (CEREC 5.0.2; Dentsply Sirona), which was pre-installed in the intraoral camera (Primescan). The crowns were then fabricated based on these virtual designs. In contrast, inLab CAD SW 19.0 (Dentsply Sirona) was used for the dies scanned with EOS. The scanned file was then converted to a DXD file to standardize the designing software with IOS. The CAD software (CEREC 5.0.2) imported the DXD file. The thickness of the crowns was set at 1.5 mm at the occlusal surface and 0.8 mm at the axial walls, with the cement spacing fixed at 60 µm. The data was sent to the milling software (CEREC 5.0.2) and the CEREC MC XL milling device (Dentsply Sirona) was used to produce the crowns. After calibrating the milling machine, 20 samples were milled from Tetric CAD blocks and the remaining 20 samples were machined using BRILLIANT Crios blocks. The crowns were installed on appropriate dies, and correct seating was verified using a sharp dental explorer under good lighting conditions and magnification. The intaglio surface of the crowns was cleaned, and any pressure spots disclosed by spraying powder (Arti-Spray®; Bausch Germany, Hainspitz, Germany) were removed with a round diamond bur.

Vertical marginal gap evaluation

Each sample was photographed using a digital microscope (Dino-Lite AM3111; AnMo Electronics Corporation, New Taipei City, Taiwan) with a built-in camera connected to an IBM-compatible personal computer at a fixed magnification of ×50. The gap width was quantified and assessed subjectively using a digital image analysis system (ImageJ 1.43u; National Institutes of Health, Bethesda, USA; https://imagej.net/ij). The marginal gap was calculated using the criteria set by Holmes et al.[16] The crowns were positioned over the matching dies. The images of the margins

Table 1. Characteristics of the investigated materials

Product	Type	Organic matrix	Inorganic filler	Manufacturer
Tetric CAD	CAD/CAM composite	Bis-GMA, Bis-EMA, TEGDMA, UDMA	barium aluminum silicate glass with a mean particle size <1 µm and silicon dioxide with an average particle size <20 nm (71.1% wt.)	Ivoclar Vivadent, Schaan, Liechtenstein
BRILLIANT Crios	CAD/CAM composite	cross-linked methacrylates (Bis-GMA, Bis-EMA, TEGDMA)	barium glass with a particle size of 1 µm and silicon dioxide with a particle size of 20 nm (70.7% wt.)	Coltène/Whaledent, Altstätten, Switzerland

CAD/CAM – computer-aided design/computer-aided manufacturing; Bis-GMA – bisphenol A-glycidyl methacrylate; Bis-EMA – bisphenol A-ethoxylated dimethacrylate; TEGDMA – triethylene glycol dimethacrylate; UDMA – urethane dimethacrylate.

were captured for each specimen. Next, using the digital image analysis system, morphometric measurements were made for each image at 28 landmarks indicated by the system throughout the cervical circumference of the specimen (6 equidistant points on the buccal and lingual surfaces, and 8 equidistant points on each proximal surface). After taking measurements for each surface, the mean value of the entire marginal gap was calculated (Fig. 1).

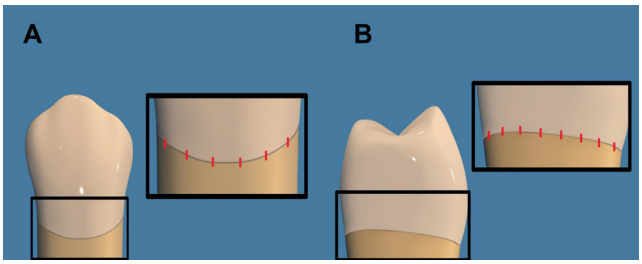

Fig. 1. Diagrammatic illustration of vertical marginal gap measurement
A – buccal aspect; B – proximal aspect.

Internal fit evaluation

The internal fit of the crown was measured using the silicone replica technique. Light-body silicon impression material (Panasil; Kettenbach, Eschenburg, Germany) was injected into the fitting surface of the crown. The crown was then placed over the printed die and pressed for 3.5 min under a load of 5 kg until the impression material was fully set according to the manufacturer's instructions. Subsequently, the crown was removed, leaving a light silicone impression on the abutment that represents the thickness of the cement space.[12] Then, the residual light impression was covered with putty silicone material (Panasil; Kettenbach) to address the challenges of cutting and managing the thin layer of the light body. Two siloxane layers were detached from the crown after setting. Using surgical blade number 15, the silicone replica was taken out and cut bucco-palatally. The handheld digital microscope was used to measure the light body thickness and assess the internal fit.[17] Prior to measurements, the microscope calibration procedures were meticulously followed. The evaluation of the internal fit included the measurements of the axial gap (AG) and the occlusal gap (OG). Eight points were measured for the buccal surface, 8 points for the lingual surface, 4 points for one occlusal slope, and 4 points for the other occlusal slope. The digital image analysis system was used to measure each point (Fig. 2).

Statistical analysis

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 27.0 (IBM Corp., Armonk, USA). The normality of the data was assessed using the Shapiro–Wilk test. Both the marginal gap and the internal fit exhibited non-parametric distribution. The Mann–Whitney *U* test was used to compare between the groups. The significance level was set at $p < 0.05$.

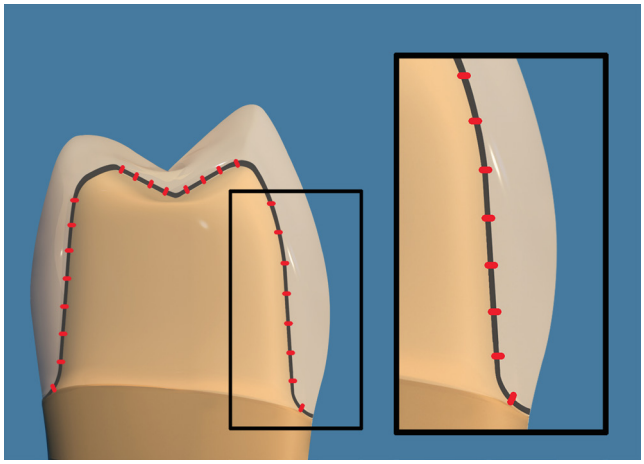

Fig. 2. Diagrammatic illustration of internal fit measurement (axial and occlusal gaps), showing measuring points for the buccal aspect, the lingual aspect and each cusp slope

Results

Marginal gap

When comparing the scanners, the results showed significant differences for both Tetric CAD and BRILLIANT Crios ($p < 0.05$) in terms of marginal gap. The marginal gap values were significantly lower for Primescan as compared to inEos X5 in both material groups ($p < 0.05$), although the difference between the Primescan Tetric CAD and Primescan BRILLIANT Crios subgroups was non-significant ($p > 0.05$). When comparing the materials within the inEos X5 group, Tetric CAD showed a significantly lower marginal gap value as compared to BRILLIANT Crios ($p < 0.05$). The marginal gap values are presented in Table 2.

Internal fit

With regard to the internal fit, both scanners showed significant differences in the Tetric CAD and BRILLIANT Crios groups ($p < 0.05$). For both tested materials, Primescan exhibited significantly lower values than inEos X5 ($p < 0.05$). When comparing the materials, no significant differences were observed for either scanner ($p > 0.05$). The internal fit values are presented in Table 3.

Table 2. Comparison of the marginal gap values [µm] in different groups

Group	Primescan	inEos X5	*Z*	*p*-value
Tetric CAD	12.0 ±8.3 (8.1–15.9)	44.8 ±14.9 (37.8–51.7)	–5.505	0.001*
BRILLIANT Crios	12.0 ±11.9 (6.5–17.5)	58.0 ±19.7 (48.8–67.2)	–5.276	0.001*
Z	0.184	–2.848	–	–
p-value	0.854	0.004*	–	–

Data presented as mean ± standard deviation (*M* ±*SD*) (95% confidence interval (*CI*)).
* statistically significant (Mann–Whitney *U* test).

Table 3. Comparison of the internal fit values [µm] in different groups

Group	Primescan	inEos X5	*Z*	*p*-value
Tetric CAD	41.8 ±22.7 (26.6–57.1)	86.0 ±49.0 (50.9–121.1)	–2.501	0.012*
BRILLIANT Crios	37.0 ±27.5 (17.3–56.7)	90.9 ±12.2 (82.7–99.1)	–3.853	0.001*
Z	0.260	1.043	–	–
p-value	0.795	0.297	–	–

Data presented as *M* ±*SD* (95% *CI*).
* statistically significant (Mann–Whitney *U* test).

Discussion

The results of the study showed that there is a statistically significant effect on the marginal and internal fit due to the differences between the acquisition systems. The intraoral scanner showed significantly better results, leading to the rejection of the null hypothesis.

Digital 3D imaging has become increasingly popular in dentistry, as it allows creating the imprints of the oral cavity. Intraoral scanners are comparable in accuracy to extraoral scanners. To evaluate their impact on the fit accuracy of the final restoration, 2 popular and readily available scanners were used in the present study.[18] To ensure consistency and a more dimensionally stable die for use during the scanning and testing of the milled crown, 3D printed resin dies were used. Additionally, to minimize difficulties while scanning larger areas, single upper premolar dies were utilized to assess the accuracy of the restorations with anatomical occlusal morphology.[19]

The longevity of the restoration is directly correlated with its precision, both internally and marginally. Good marginal adaptation helps prevent cement disintegration, and subsequent cavities and discoloration. Improved internal adaptability may increase the resistance and retention of the restoration. Thus, to verify the accuracy of scanners, measurements of marginal and internal fit were taken.[20,21]

There are various methods to test and measure the marginal gap, but the direct view method using a digital microscope is considered the most practical, accurate, quick, and easy way to determine the gap distance. In addition, unlike with the cementation, embedment and sectioning methods, which destroy the crown, the crown can be recovered.[22] In the present study, the marginal accuracy of the crown was determined by calculating the vertical difference between the margin of the die and the margin of the monolithic crown without cementation. After cementation, factors such as the cement type, cement viscosity and the cementation technique can affect the accuracy of the primary adaptation, potentially increasing the marginal discrepancy.[23] In addition, this non-destructive method has been previously used in dental studies.[24–26] Similarly, various techniques have been employed to evaluate the internal fit of restorations. In the present study, the silicon replica technique, a prevalent non-destructive method for in vitro internal fit evaluation, was utilized. The technique is known for its simplicity and efficiency.[21] Furthermore, a 50-newton force was applied to the crowns, using a unique mechanism to ensure uniformity and eliminate potential data variability.

In our study, the average gap obtained when using the Primescan system was significantly lower than in the case of scanning with inEos X5. This result is consistent with the findings of Zimmermann et al.[27] and Nulty,[28] who reported significantly higher accuracy with Primescan as compared to other scanning systems. Malaguti et al. determined that the marginal and internal fit in the case of intraoral scanners were significantly better in comparison with laboratory scanners.[29] However, these results contradict the findings of Lee et al., who reported that extraoral scanners were more accurate than intraoral scanners.[30] Conversely, Da Costa et al. did not find any difference in the marginal gap of restorations when the optical impression was taken either intraorally or extraorally.[31]

Primescan uses structured light–confocal microscopy with a high-precision Smart Pixel Sensor that evaluates the contrast of each pixel at a high resolution. Primescan provides an exceptional level of scanning precision by combining over 50,000 photos and capturing up to 1,000,000 3D points per second for each 3D image. The patent scanning principle consists in using an optical high-frequency contrast analysis to calculate 3D points, which results in increased accuracy.[27,28,32] On the other hand, inEOS X5 is a blue light scanner with a narrow wavelength, which enables better filtering of interference from ambient light and improved scanning repeatability.[33]

The marginal and internal fit of the crowns produced using the CAD/CAM technology were within the clinically acceptable ranges. The mean marginal gap ranged from 12.0 µm to 58.0 µm, and the internal gap varied between 37.0 µm and 90.9 µm. Clinically acceptable marginal and internal fit results are the outcome of the full CAD/CAM process, which includes milling and scanning. It has been found that the 4-axis milling machine used in the CAD/CAM systems under investigation provides better results in terms of internal adaptation of the milled restorations. Rotating the milling spindle with a higher number of milling axes may improve the accuracy of the milling machine. This may be related to improved finishing in the cervical region of the restoration, which in turn can affect marginal adaptation.[19]

Although the internal fit is clinically less significant than the marginal fit, it still affects the durability of the crown. To ensure the proper crown seating, resistance and retention, the internal fit of the crown must be appropriate.[34]

The CAD/CAM technology can potentially improve the mechanical behavior and marginal integrity of tooth restoration systems when using restorative materials with a low elastic modulus. These materials are more resilient and machinable due to their lower Young's modulus, and are less prone to chipping and fractures. Despite their clinical

advantages, they may be a subject of recurring deterioration combined with microleakage, and the consequent restorative failure caused by repetitive elastic deformation at the margins. This result could be attributed to differences in the mechanical characteristics, chemical composition and microstructure of the studied CAD/CAM restorative crowns. Crowns that are milled from blocks and disks produced industrially at high temperatures and pressures exhibit increased filler volume fractions and conversion rates.[35] This study selected Tetric CAD and BRILLIANT Crios, as they revealed superior mechanical properties in comparison with other resin composite ceramic materials,[9] with little data about their fit and marginal adaptation.

Although the extraoral scanner showed higher marginal gap and internal fit values than the intraoral scanner, it was concluded that scanners using blue light, including inEos X5, are accurate.[36] The ceramic material could also have an impact on the marginal accuracy of the crowns created using the CAD/CAM technology.

Limitations

There are some limitations to this research. The reflectance of the 3D printed dies differed from that of natural teeth. Additionally, scanning a single die without the presence of the neighboring teeth is another limitation. Moreover, the absence of complex environmental factors, such as patient movement, saliva, limited space, the presence of blood and gingival crevicular fluid (GCF), and humidity, when using the intraoral camera, as well as the lack of an impression or a model when employing the extraoral camera, are also considered the limitations of the study. More research is required to determine the optimal cement space value for the CAD design. This is crucial for maximizing retention and resistance, improving clinical outcomes and minimizing the marginal gap without compromising the internal fit. In vivo investigations are required to accurately simulate the clinical situation.

Conclusions

The crowns scanned using Primescan and milled with the MC XL milling machine showed better internal fit and marginal accuracy. The intraoral scanning approach was found to be superior in influencing the vertical marginal gap distance and internal fit of monolithic crowns. However, the marginal accuracy and internal fit shown by the extraoral scanner were also within the clinically acceptable ranges. Furthermore, the examined monolithic crowns displayed marginal gap distance and internal fit values that were within the accepted clinical limits.

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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Original paper

Influence of dental implants on the accuracy of measuring the postoperative labial alveolar bone thickness in the maxillary anterior region: An in vivo CBCT study

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Abstract

Background. The accuracy of cone-beam computer tomography (CBCT) in measuring the labial alveolar bone thickness requires further evaluation.

Objectives. The aim of the present study was to evaluate the impact of dental implants on the accuracy of CBCT in measuring the postoperative labial alveolar bone thickness in the maxillary anterior region.

Material and methods. The distance from the labial alveolar bone surface at the bone crest level to the implant neck was measured using 2 methods. One method involved using periodontal probes during an immediate implant surgery, while the other employed the CBCT scans obtained immediately post-operation.

Results. Twenty patients were recruited from the Department of General Dentistry at the Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, China, from February 2023 to October 2023. In total, 20 implants with a diameter of 3.3 mm were placed surgically. The average distance from the labial alveolar bone surface at the bone crest level to the implant neck in the group of 20 patients, obtained through the intraoperative measurement, was 3.7 ±0.8 mm. The corresponding average value based on the CBCT data of the 20 patients was 3.1 ±0.6 mm. A significant difference was observed between the 2 methods ($p < 0.001$). The average diameter of the 20 implants measured using the CBCT scans was 4.1 ±0.4 mm, which was significantly greater than the actual implant diameter of 3.3 mm.

Conclusions. The distance from the labial alveolar bone surface at the bone crest level to the implant neck, which comprises the thickness of the labial alveolar bone and the jumping gap, was smaller according to the CBCT data than the actual values obtained from intraoperative measurements. However, the average diameter of the 20 implants measured using the CBCT scans exceeded the actual implant diameter of 3.3 mm. When assessing the thickness of the labial alveolar bone around implants in the maxillary anterior region using CBCT during follow-up, a moderate underestimation of the labial bone thickness may occur.

Keywords: CBCT, measurement method, immediate implantation, labial alveolar bone thickness

Highlights

- The CBCT-measured distance from the labial alveolar crest surface to the implant neck, including the labial alveolar bone thickness and the jumping gap, was smaller than the actual intraoperative measurement.
- The CBCT-measured mean diameter of the 20 implants was greater than the actual implant diameter of 3.3 mm.
- Follow-up assessments based on CBCT may moderately underestimate the labial alveolar bone thickness surrounding dental implants.

Introduction

Immediate implantation in the maxillary anterior region has been gaining increasing attention.[1] A growing number of studies now focus on achieving stable and satisfactory outcomes in immediate implant placement.[2–4] The advantages of immediate implantation are manifold, including shortening the edentulous period, reducing the number of surgical procedures and preserving an intact extraction socket.[5] The presence of complete socket walls also simplifies and facilitates the placement of low-substitution bone graft materials within the jumping gap.

The thickness of the labial alveolar bone surrounding dental implants is a critical determinant of both alveolar bone stability and esthetic outcomes.[6] It can also serve as a predictor of esthetic results and potential complications.[7,8] However, improper three-dimensional (3D) implant positioning may result in labial alveolar bone loss, thereby compromising the stability and esthetics of the final restoration.[9]

Ideal implant placement in the maxillary anterior region requires precise positioning in the mesiodistal, buccolingual and apicocoronal dimensions, along with correct angulation. To guide this process, the proposed assessments categorize implant sites into 'comfort' and 'danger' zones.[10] In the buccolingual dimension, implants should be positioned 1 mm palatal to an imaginary line drawn through the emergence profile of the adjacent teeth.[10]

To fulfill the requirements for immediate implantation, the ideal implant position is adjacent to the palatal bone wall of the alveolar socket, which naturally creates a jumping gap between the implant and the labial bone wall. In CBCT images, however, the thickness of the labial alveolar bone surrounding the implant comprises both the labial socket wall and the jumping gap, as this gap is tightly packed with a non-absorbable bone graft material. New bone formation occurs as the osteoblasts originating from the alveolar socket migrate into the graft material, although some resorption of the labial socket wall may occur simultaneously. In this study, the labial alveolar bone thickness of the implant is defined as the distance from the labial bone surface at the bone crest level to the implant neck.

Studies have demonstrated that the postoperative thickness of the labial bone has a significant impact on the success of implant restorations.[11] An insufficient labial bone thickness around the implant is associated with an increased risk of peri-implant marginal bone loss, and marginal bone loss exceeding the limits of physiological remodeling is considered a diagnostic criterion for peri-implant disease.[12,13] Currently, the assessment of the labial bone thickness around implants relies primarily on CBCT.[14,15] In addition, CBCT plays an increasingly important role in the customization of implant surgery and in clinical decision-making for immediate implantation by enabling the evaluation of the sagittal root position of maxillary anterior teeth.[16,17] However, discrepancies have been noted between CBCT interpretations and intraoperative findings. Several studies have reported an apparent increase in the implant diameter in CBCT in vitro, as well as the underestimation of the labial bone thickness in fresh-frozen human cadaver heads.[18,19]

To evaluate the precision of the CBCT measurements of the postoperative labial alveolar bone thickness, the present study compared the intraoperative measurements obtained during immediate implant placement with the corresponding postoperative CBCT data. This comparative analysis will help improve the accuracy of monitoring the actual labial bone thickness around implants during follow-up using CBCT.

Material and methods

Study design and participants

This study is an in vivo CBCT agreement study evaluating the measurement of the postoperative labial alveolar bone thickness in the maxillary anterior region. From February 2023 to October 2023, patients presenting with dental trauma that resulted in teeth deemed to have a hopeless prognosis, willing to undergo implant restoration, were recruited from the Department of General Dentistry at the Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, China. The inclusion criteria were as follows: (1) age ≥18 years; (2) indication for the traumatic extraction of maxillary ante-

rior teeth; (3) mouth opening greater than 30 mm; and (4) stable occlusion of the proximal and mesial/distal teeth adjacent to the affected site. The exclusion criteria were as follows: (1) insufficient distance between the implant site and the alveolar socket walls; (2) defects in the labial bone plate; and (3) untreated severe caries or uncontrolled periodontitis in the adjacent teeth. The study was approved by the Ethics Committee of the Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, China (SH9H-2022-T358-1). All patients signed informed consent forms. The research was conducted in accordance with the Declaration of Helsinki.

After screening patients according to the inclusion and exclusion criteria, eligible participants were informed about the purpose and procedures of the study, and subsequently provided written informed consent. All implant surgeries were performed by the same experienced surgeon in accordance with each patient's treatment plan.

The surgical procedure was conducted in the following steps. Patients first rinsed their mouths with a 2.5% iodophor intraoral antiseptic for 1 min. Standard surgical disinfection protocols were then performed, and sterile drapes were applied. Under local anesthesia (Primacaine™; Acteon, Merignac, France), a microcrestal flap technique was used. A crevicular incision was made to create a micro-flap, and a full-thickness flap was elevated to expose the cervical areas of the adjacent teeth and the alveolar crest at the extraction site. Minimally invasive tooth extraction was carried out under direct visualization. After verifying the integrity of the alveolar bone walls, a 3.3-millimeter bone-level titanium (Ti) implant (SLA type; Straumann Group, Basel, Switzerland) was placed into the extraction socket.

Intraoperative measurements were obtained using a periodontal probe and documented with photographs. Bio-Oss® Collagen (Geistlich Pharma, Wolhusen, Switzerland) was compactly placed into the jumping gap. A CBCT scan (ProMax® 3D; 96 kV, 5.6 mA, exposure time: 12.094 s, voxel size: 0.2 mm; field of view (FOV): 13.0 cm × 9.0 cm; Planmeca, Helsinki, Finland) was performed immediately after implant placement.

The study workflow is shown in Fig. 1.

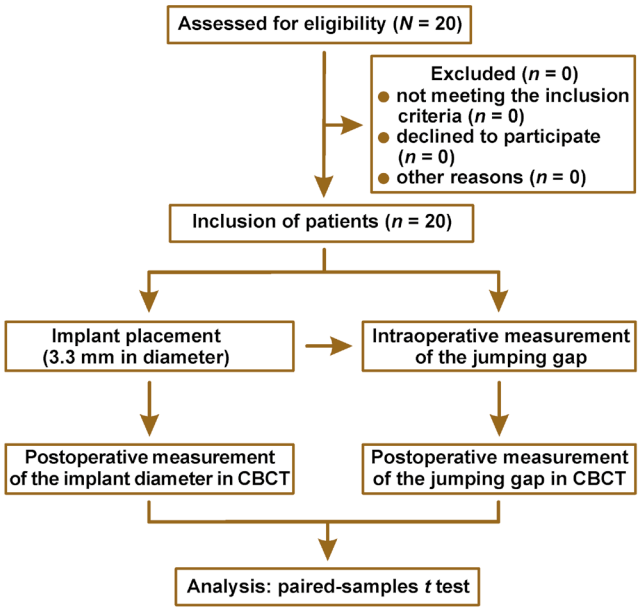

Fig. 1. Study workflow diagram

Intraoperative measurement of the labial alveolar bone thickness of the implant

The distance from the labial bone surface at the bone crest level to the implant neck was measured intraoperatively using a standard periodontal probe. To ensure measurement accuracy, the probe was calibrated with the same steel ruler before each use. Photographs were then taken and the corresponding distance in the images was measured using the ImageJ software (https://imagej.net/ij), as shown in Fig. 2. All measurements were independently performed by 2 trained assessors to minimize the observer bias.

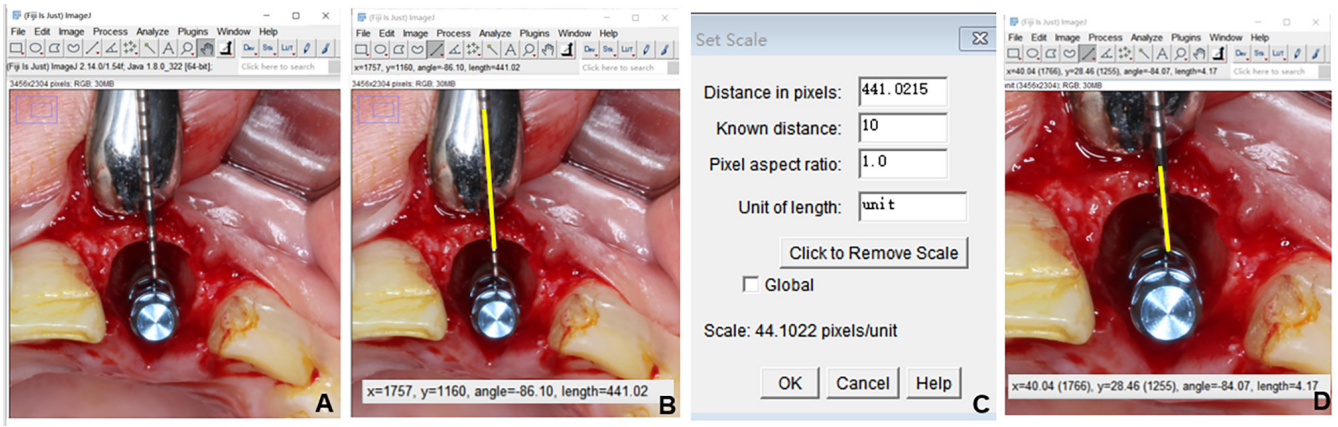

Fig. 2. Intraoperative measurement of the labial alveolar bone thickness of the implant using ImageJ

A – measurement of the labial alveolar bone thickness of the implant with a periodontal probe; B – counting the number of pixels in the measurement image; C – conversion of the pixel count into the actual distance; D – measurement of the labial alveolar bone thickness.

Postoperative measurement of the labial alveolar bone thickness of the implant with the CBCT data

The SmartVPro software, v. 2.1.1.4895 (LargeV, Beijing, China), equipped with a proprietary linear measurement tool, was used to measure the labial alveolar bone thickness and the implant diameter in the multiplanar reconstruction (MPR) CBCT scans obtained immediately postoperatively. The focal planes of the CBCT scans were adjusted to the center of the implant in both the mesiodistal and buccolingual dimensions,[20] with the oblique sagittal view oriented perpendicular to the dental arch.

In the CBCT images, the labial alveolar bone thickness – comprising the labial socket wall and the jumping gap filled with Bio-Oss Collagen – was measured at the cervical level of the implant, as illustrated in Fig. 3. All measurements were independently performed by 2 trained assessors to minimize the observer bias.

Statistical analysis

The measurement data was statistically analyzed using IBM SPSS Statistics for Windows, v. 21.0 (IBM Corp., Armonk, USA). All data is presented as mean ± standard deviation ($M \pm SD$). The one-sample Kolmogorov–Smirnov test was used to assess normality. Depending on the distribution characteristics, the one-sample t test and the paired-samples t tests were performed for statistical comparisons. A significance level of $p < 0.05$ was considered statistically significant.

Results

A total of 20 patients were included in the study, with a mean age of 34.7 years (range: 23–53 years). The cohort consisted of 8 males and 12 females. In total, 20 implants were placed in the maxillary anterior region, including 15 Straumann® bone-level tapered implants and 5 Straumann® bone-level implants. The participants' demographic and clinical data is summarized in Table 1.

Measurement of the labial alveolar bone thickness of the implant

No statistically significant difference was observed between the intraoperative measurements of the distance from the labial alveolar bone surface at the bone crest level to the implant neck obtained by 2 trained assessors using

Table 1. Study participants' characteristics

Patient No.	Gender	Age [years]	Tooth
1.	M	39	11
2.	M	34	21
3.	F	34	11
4.	F	34	21
5.	M	26	12
6.	F	23	12
7.	F	25	11
8.	M	25	11
9.	F	28	11
10.	F	34	12
11.	F	36	22
12.	F	45	21
13.	M	46	13
14.	F	36	12
15.	F	37	12
16.	M	41	12
17.	F	53	11
18.	M	46	22
19.	F	27	21
20.	M	43	22

M – male; F – female.

Fig. 3. Cone-beam computed tomography (CBCT) measurement of the implant diameter and the labial alveolar bone thickness

A – schematic diagram of an immediate implant surgery; B – focal planes for determining the measurement sites; C – measurement of the labial alveolar bone thickness of the implant and the implant diameter.

a standard periodontal probe (t = 1.67; p = 0.112). These measurements are summarized in Table 2. The mean intraoperative distance for the 20 patients was 3.7 ±0.8 mm.

Similarly, no significant difference was found between the labial alveolar bone thickness measurements obtained from CBCT by the 2 assessors (t = 0.94; p = 0.360), as shown in Table 3. The mean labial alveolar crest thickness measured in immediate postoperative CBCT was 3.1 ±0.6 mm.

A significant difference was observed between the intraoperative measurements and the CBCT measurements in the immediate postoperative period (t = 4.85; p < 0.001). There was a positive correlation between the 2 sets of measurements (r = 0.726; p < 0.001). The results are presented in Fig. 4.

Table 2. Mean labial alveolar bone thickness of the implant [mm], as measured intraoperatively with a standard periodontal probe

Patient No.	1st assessor	2nd assessor
1.	4.29	4.17
2.	4.27	4.18
3.	2.85	3.10
4.	3.75	4.12
5.	4.47	5.05
6.	2.84	2.98
7.	5.37	5.21
8.	3.48	3.74
9.	3.13	3.05
10.	4.03	3.94
11.	3.37	3.41
12.	3.04	3.11
13.	4.89	4.79
14.	2.71	2.85
15.	2.46	2.45
16.	3.54	3.70
17.	2.92	2.96
18.	3.47	3.53
19.	4.98	4.77
20.	3.03	3.25

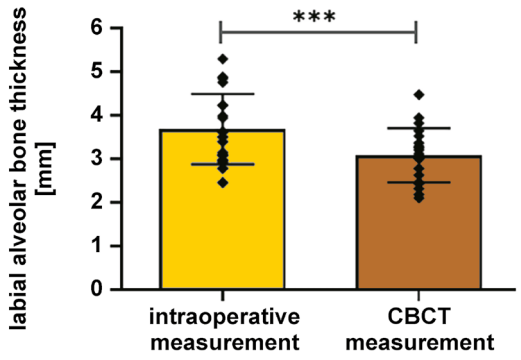

Fig. 4. Results of the intraoperative and cone-beam computed tomography (CBCT) measurements of the labial alveolar bone thickness of the implant for 20 patients

The intraoperative measurement with a standard periodontal probe: measuring the distance from the labial alveolar crest surface to the implant cervix.
*** highly statistically significant (p < 0.001).

Implant diameter measurement

The mean diameter of the 20 implants measured by CBCT was 4.1 ±0.4 mm. The actual diameter of the implant was 3.3 mm. There was a statistically significant difference (t = 8.17; p < 0.001) between the diameters measured in the CBCT images and the actual value. The results are presented in Fig. 5.

Table 3. Mean labial alveolar bone thickness of the implant [mm], as measured in the cone-beam computed tomography (CBCT) images immediately postoperatively

Patient No.	1st assessor	2nd assessor
1.	3.71	3.57
2.	2.75	1.89
3.	2.64	3.86
4.	3.59	2.81
5.	3.78	4.10
6.	2.97	1.89
7.	3.35	3.37
8.	3.84	3.20
9.	2.86	2.07
10.	2.83	3.74
11.	3.33	2.90
12.	2.68	2.57
13.	3.96	3.67
14.	2.22	1.98
15.	2.07	2.29
16.	2.98	2.57
17.	3.10	3.04
18.	2.88	3.12
19.	4.30	4.65
20.	2.95	3.06

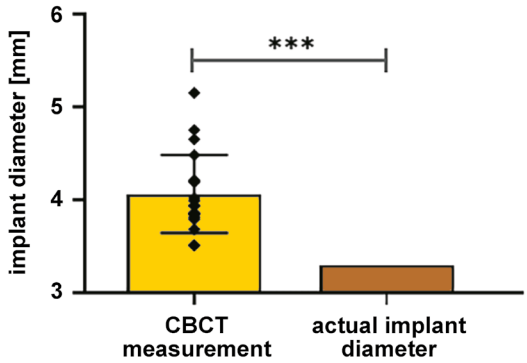

Fig. 5. Results of the cone-beam computed tomography (CBCT) measurements of the implant diameter for 20 patients in comparison with the actual implant diameter of 3.3 mm

*** highly statistically significant (p < 0.001).

Discussion

Immediate implantation offers the advantages of reducing the duration of edentulousness and minimizing the number of clinical visits.[11] As reported in the literature, over a 5-year follow-up period, no significant differences were observed between immediate and delayed implants regarding clinical outcomes, including the implant success rates and the preservation of the alveolar bone volume.[5,21] Additionally, clinical evidence suggests that patients with a thin gingival phenotype or a minor loss of the labial lateral bone plate can achieve stable restorative outcomes over long-term follow-up with immediate implant placement.[22] The effective management of both peri-implant soft and hard tissues is crucial for ensuring the predictable success of immediate implants.[23–25]

Achieving an ideal 3D implant position is one of the most critical factors for ensuring long-term functional and esthetic outcomes in implant restorations. The thickness of the labial bone at the implant neck after immediate implantation is a key predictor of implant prognosis.[26] Evidence shows that in the maxillary anterior region, when the implant neck lies less than 1.5 mm from the margin of the labial bone, greater vertical bone resorption can occur, accompanied by a reduction in the width of keratinized gingiva and decreased resistance to peri-implantitis.[27] Understanding the pattern of bone resorption is therefore essential. Peri-implant buccolingual bone resorption occurs predominantly about 1 mm apical to the implant platform, with the degree of resorption gradually diminishing toward the apex. Moreover, after immediate implantation, the labial alveolar bone wall is particularly susceptible to resorption – more so than the palatal wall – due to lip muscle pressure. Thus, the labial alveolar bone thickness can be regarded as a critical indicator for predicting the long-term success of implant restorations.[28]

With the widespread adoption of CBCT, it has become an essential diagnostic imaging tool in implant treatment. Cone-beam computed tomography enables the 3D visualization of the anatomical morphology of the alveolar bone and the peri-implant structures. However, artifacts – the virtual distortions produced during image reconstruction – can compromise image quality by reducing contrast between the adjacent structures.[21] Among these, metal artifacts exert the greatest influence. Since CBCT operates at lower energy levels than the conventional computed tomography (CT), it generates more pronounced artifacts, which in turn affect the visibility of peri-implant tissues and measurement accuracy.[29] Evidence also indicates that CBCT is less sensitive in detecting small areas of peri-implant bone loss.[30] In this study, all implants used were 3.3 mm in diameter, yet the implant diameters measured on postoperative CBCT averaged 4.1 mm, which constituted a statistically significant difference. This discrepancy may be attributable to metal artifacts and the cupping artifact associated with the cylindrical geometry of the implant.[29] Recent studies further report that Ti implants produce fewer artifacts than zirconia implants, and that artifact intensity can be reduced by increasing the CBCT tube voltage or by applying data processing algorithms that reduce metal artifacts.[31]

A study conducted on fresh-frozen human cadaver heads reported a 15% increase in implant diameter.[19] In the present study, a blooming percentage of up to 24% was observed, which has important implications for assessing the labial bone thickness in CBCT. In living subjects, the soft tissues surrounding the implant, as well as moisture within bone and blood, can absorb X-rays, potentially influencing the CBCT grayscale values and measurement accuracy.[32,33] The presence of soft tissues and the absence of moisture changes typically introduced by freezing and thawing may further contribute to a greater underestimation of the labial bone thickness in vivo.

Although diagnosing peri-implant pathology based on the radiographic assessment of the bone plate thickness is inherently challenging, especially at the early stages of disease progression,[34] the evaluation of the labial bone plate thickness using CBCT after implant placement remains a critical component of postoperative follow-up. However, current studies have insufficiently addressed the accuracy of CBCT and the factors that may influence the reliability of its measurements.[35] In vitro research has shown that CBCT detects labial bone plates of ≤0.5 mm around implants at a rate of less than 20%; yet, for every 1-mm increase in the bone plate thickness, the detection rate increases by 30.6%.[23] In the present study, the CBCT measurements demonstrated a 0.6-mm reduction in the labial alveolar bone thickness of the implant as compared to the intraoperative measurements, indicating a pronounced tendency for CBCT to underestimate the true labial bone thickness. This finding aligns with previous in vitro studies using ribs and chilled skulls.[24,35] Such underestimation may be attributable to the metal artifacts generated by the implant, as well as the influence of the surrounding anatomical structures. Notably, this tendency becomes further amplified when the labial bone thickness is less than 1 mm.[36]

This study suggests that when the labial alveolar bone thickness around implants in the maxillary anterior region is assessed using CBCT during follow-up, a moderate overestimation of the buccal bone thickness is acceptable. Therefore, implant stability should be evaluated promptly using complementary methods, such as the implant stability quotient (ISQ) and the patient's subjective perception, to ensure accurate clinical judgment.

Limitations

Owing to the relatively small sample size and the limited variability in implant types, the findings of this study have certain limitations. To reduce bias and enhance external validity, future research should include multicenter

studies with larger sample sizes and incorporate implants from different manufacturers to better observe radiographic variations across implant systems.

Conclusions

Within the limitations of the present study, the labial alveolar bone thickness of the implants in the maxillary anterior region measured in CBCT was consistently lower than the intraoperative values. In contrast, the mean implant diameter measured in CBCT exceeded the actual implant diameter of 3.3 mm. Therefore, when assessing the labial alveolar bone thickness around implants in the maxillary anterior region during follow-up, a moderate overestimation of the CBCT-derived values is permissible.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, China (SH9H-2022-T358-1). All patients signed informed consent forms. The research was conducted in accordance with 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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Original paper

Navigating trends, collaborations and future directions in the odontogenic lesion literature: 40-year bibliometric mapping analysis

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Abstract

Background. The oral and maxillofacial region comprises a wide spectrum of pathological conditions, among which odontogenic lesions (OLs) are particularly prevalent. Ongoing academic research in this field focuses on advancing diagnostic modalities, improving therapeutic strategies and elucidating disease pathogenesis, thereby promoting interdisciplinary collaboration across medicine, pathology and dentistry.

Objectives. Within this context, the present bibliometric analysis aimed to evaluate the current landscape of OL research, retrospectively map the field, and identify the emerging trends and future research directions.

Material and methods. Guided by the principles of the Leiden Manifesto, this comprehensive bibliometric study analyzed 4,298 publications published between 1980 and 2023, and retrieved from the Web of Science Core Collection. The analysis examined international collaboration patterns, keyword evolution, institutional affiliations, co-citation networks, and thematic clustering. CiteSpace, RStudio Bibliometrics and VOSviewer were employed to explore bibliometric relationships and generate visualizations.

Results. The 40-year bibliometric mapping analysis of OL research revealed a diverse and evolving scientific landscape. A total of 4,298 publications encompassing 40,209 references demonstrated extensive international collaboration, represented by 111 nodes and 385 collaborative links. India emerged as the most prolific contributor (670 publications), while the USA led in citation impact (12,169 citations). Co-citation analysis identified highly influential works, notably updates to the World Health Organization (WHO) classifications. Thematic clustering highlighted major research domains, including odontogenic keratocysts, ameloblastomas and calcifying odontogenic lesions. Trend analysis further indicated a shift from traditional pathological concepts toward advanced diagnostic approaches, with growing emphasis on gene expression profiling and imaging technologies, such as cone-beam computed tomography (CBCT).

Conclusions. In the present study, the historical development of OL literature was evaluated using bibliometric analysis, identifying the most influential publications, major thematic domains and research hotspots. The emerging trends in OL research continue to shape the field and drive advances in both scientific understanding and clinical practice.

Keywords: oral pathology, bibliometric analysis, odontogenic cysts, odontogenic tumor, medical bibliography

Highlights

- This 40-year bibliometric analysis reveals expanding global research on odontogenic lesions (OLs), with a sharp increase in collaboration post-2000.
- India is the most prolific country in terms of OL research, while the USA has the highest citation impact, indicating differing productivity and influence.
- Co-citation and clusterig analyses highlight World Health Organization (WHO) classification updates as the major factors shaping current research on OLs.
- Thematic evolution shows a transition from histopathology to advanced diagnostic methods, like molecular profiling and cone-beam computed tomography (CBCT).
- The findings provide a roadmap of past development and current trends, guiding future clinical and academic research on OLs.

Introduction

The oral and maxillofacial region encompasses a wide spectrum of pathological conditions of diverse etiology.[1] Dental caries, oral lesions and odontogenic lesions (OLs) are among the most common disorders affecting this region.[2,3] Dental caries is a multifactorial disease initiated by microbiological shifts within the complex oral biofilm, and influenced by factors such as salivary flow and composition, fluoride exposure, dietary sugar intake, and preventive behaviors.[2,4] Oral lesions are defined as abnormal or pathological alterations of the tissues within the oral cavity and the associated structures, presenting as changes in color, texture or surface appearance. These lesions may arise from a variety of causes, including infection, inflammation, trauma, or systemic disease.[3,5]

Within this spectrum, odontogenic lesions (OLs) are particularly prevalent.[6] According to the World Health Organization (WHO), OLs are pathological conditions associated with tooth development or tissues of dental origin, and commonly occur within the jaw bones.[7] Odontogenic tissues develop through time-dependent, tightly regulated interactions between epithelial and mesenchymal components, giving rise to a wide spectrum of morphological patterns in the lesions derived from these tissues.[8] Although most OLs are benign, they exhibit considerable clinical and histopathological diversity, and represent an important entity in clinical practice.[7] In the oral and maxillofacial region, OLs typically arise from the abnormalities occurring during embryonic tooth development. These conditions may manifest in various forms, including jaw bone masses, dental dysplasia, or other dental anomalies.[9] The stimuli initiating odontogenic epithelial growth and the subsequent lesion development remain poorly understood.[10] Clinically, OLs most often present as painless masses; however, depending on their size and anatomical location, they may exert pressure on the adjacent structures, leading to functional impairment or esthetic concerns.[11]

Odontogenic lesions play a significant role in dentistry, pathology, oral surgery, and radiology.[12] At the academic level, ongoing research continues to expand knowledge on OLs through the investigation of novel diagnostic modalities, therapeutic strategies and the underlying pathogenetic mechanisms. Such studies enhance the understanding of these lesions, and contribute to improved clinical management and patient care. Moreover, academic research on OLs promotes interaction and collaboration among the disciplines of medicine, pathology and dentistry. A multidisciplinary approach to the evaluation and management of these lesions enables more comprehensive patient care, and fosters continuous advancement in this field. In this context, a thorough and critically appraised review of the available scientific literature is essential for informed clinical decision making.

In 1977, Dr. E. Garfield pioneered bibliometric studies by identifying highly cited publications to investigate the factors underlying their citation impact and influence within a given field.[13] Bibliometric analysis is a quantitative method used to evaluate the productivity, impact and evolving trends of a scientific discipline by analyzing the characteristics of selected publications within specific databases, including citation counts, co-citation patterns, and the year of publication.[14] The cumulative nature of scientific knowledge, global advances in health systems, and the growing number and diversity of academic journals have contributed to a substantial increase in the published research. Despite this growth, there remains a lack of quantitative and objective evaluations identifying the publication patterns related to OLs within the field of oral and maxillofacial surgery. A comprehensive bibliometric analysis in this area would enable researchers to identify research hotspots, assess influential contributions, and gain insight into current perspectives and the emerging trends.

The aim of this study was to analyze and visualize the existing body of literature on OLs in order to retrospectively map the field and identify potential directions for future research.

Material and methods

This study was conducted in accordance with the principles set forth in the Leiden Manifesto. Bibliometric analysis is exempt from the institutional ethics committee review, as it relies solely on publicly available electronic sources and does not involve the generation of novel data or the use of private patient information.

Articles were retrieved from the Web of Science Core Collection (WoS-CC) database on the same day (December 2, 2023) to avoid bias due to daily database updates. The literature was filtered for the period between 1980 and 2023. Medical Subject Headings (MeSH) were used to select the following search terms: "ALL=("odontogenic" and ("lesion" or "cyst" or "tumor" or "pathology" or "malignity") not ("nonodontogenic")) and Dentistry Oral Surgery Medicine or Pathology or Surgery or General Internal Medicine (Research Fields) and Soft Tissue, Bone & Nerve Cancers or Dentistry & Oral Medicine or Cosmetic Surgery or Molecular & Cell Biology – Cancer & Development (Citation Topics Meso) and Article, Review Article or Book (Document Types)". No language restrictions were applied. Articles unrelated to oral and maxillofacial surgery were excluded (this exclusion was done through the Research Fields and Citation Topics Meso filters).

A list of articles was compiled in a Microsoft Excel spreadsheet (Microsoft Corporation, Redmond, USA), and the following information was recorded: journal name; citation rank; citation density (citations per year); first author's name; year of publication; first author's institution and country of origin; study type; study design; research areas; keywords; author keywords; and indexing information. The finalized dataset was imported into CiteSpace (v. 6.2.R6; Drexel University, Philadelphia, USA), RStudio (v. 4.3.2; Posit, Boston, USA) and VOSviewer (v. 1.6.20; Leiden University, the Netherlands) for statistical analysis and the generation of maps and graphs. CiteSpace and VOSviewer were used to examine relationships among authors, institutions, countries, and keywords, while co-citation analysis was conducted to identify common research foci and the emerging hotspots. Descriptive statistics were used to summarize study characteristics. The analysis excluded the examinations of author self-citations and H-index values, as these metrics are database-specific and not consistently available across sources.

Results

Overall results

A total of 4,298 publications and 40,209 references were retrieved from the WoS-CC database to investigate the basic publication characteristics, hotspots and the boundaries of this research area (Table 1). A total of 373 sources (journals, books, etc.) were identified. The average number of citations per article was 13.73. The average logistic annual growth rate (AGR) with regard to the number of publications was 7.01% (Fig. 1). The search retrieved a total of 4,304 articles, of which 6 were excluded. A total of 4,298 articles were included in the analysis (Fig. 2).

Collaboration across countries

In terms of cooperation across countries, the nodes and links were created using CiteSpace. A total of 111 nodes and 385 links were identified (Fig. 3). The size of the nodes indicates the frequency of co-citation, and the links between the nodes indicate connections between co-citations. Nodes of different colors represent specific years. India was the top publishing country with 670 publications, followed by the USA (609), Brazil (536), Japan (462), China, and Italy. The top 5 countries in terms of centrality (the purple circle), which reflects the extent to which a country functions as a bridging node within international collaboration networks rather than the publication volume alone, were the USA (0.59), India (0.24), the UK (0.15), Denmark (0.13), and Germany (0.12). The MCP index (multiple-country publications) illustrates the number of documents with at least one co-author from a different country for each respective country, thus measuring the extent of international collaboration within that country (Fig. 4). In the visualization based on the nationality of authors in the dataset, India (2,057), Brazil (1,996) and the USA (1,714) emerged as the leading nations. The color intensity deepens as the number of publicatıons increases (Fig. 5).

Table 1. General outcomes

Main characteristics		Outcomes
Timespan		1980–2023
Sources (journals, books, etc.)		373
Documents		4,298
AGR [%]		7.01
Document average age [years]		11.5
Average number of citations per document		13.73
References		40,209
Authors		12,969
Authors of single-authored documents		146
International co-authorship [%]		12.77
Average number of co-authors per document		4.44
Document types	original article	3,688
	review	610

AGR – annual growth rate.

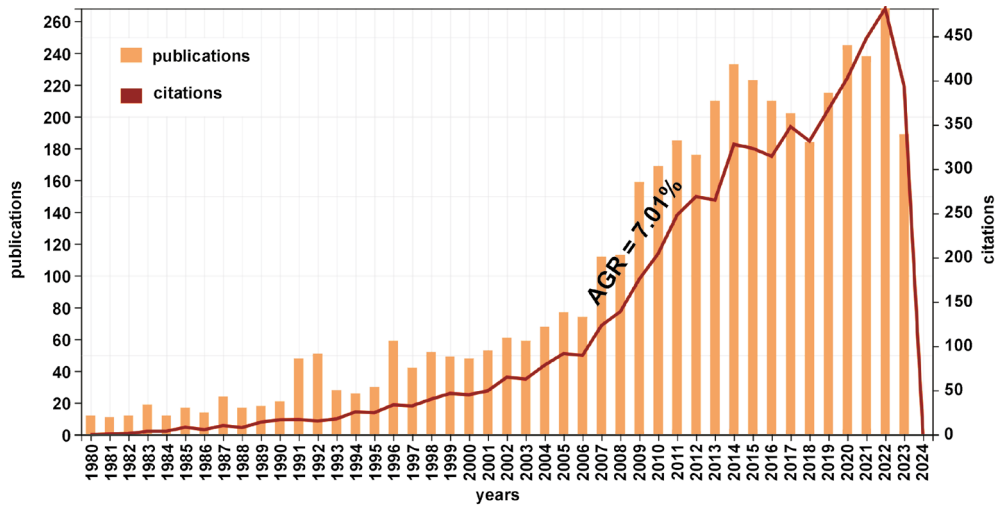

Fig. 1. Number of publications on odontogenic lesions (OLs) related to the number of citations by year
Taken from the Web of Science Journal Citation Reports (WoS-JCR).

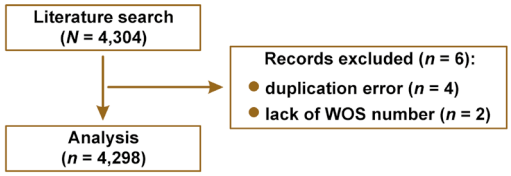

Fig. 2. Flowchart of the study
WOS – Web of Science.

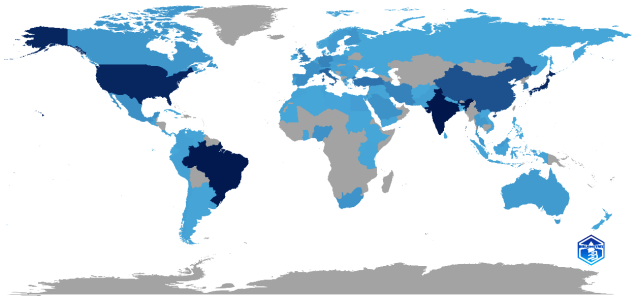

Fig. 5. Scientific production of the countries

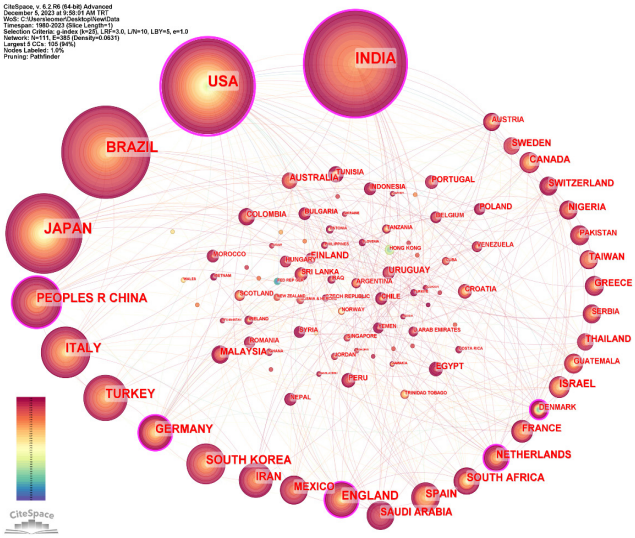

Fig. 3. Cross-country collaboration map

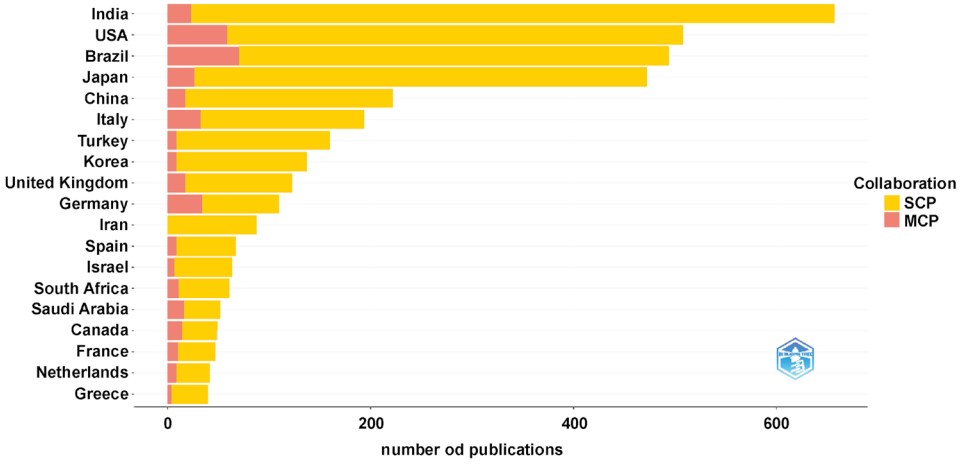

Fig. 4. Corresponding authors' countries
SCP – single-country publications; MCP – multiple-country publications.

Keyword analysis

In Fig. 6, the WordCloud illustrates a network of keywords derived from the cited literature. Among the KeyWord Plus terms extracted from the titles of the articles, a clear distribution can be observed around the most prominent keyword "expression" (338). Other frequently occurring terms include "tumor", "lesion", "odontogenic tumors", and "ameloblastoma", each appearing 199 times or more. In the visualization, the font size reflects the frequency of each keyword, while more central placement indicates greater relevance within the overall research theme.

The construction of a keyword co-occurrence network, as shown in Fig. 7, enables the exploration of the conceptual structure of the research domain. This analysis examined relationships among author-assigned keywords based on the frequency and repetition of their co-occurrences. When a minimum co-occurrence threshold of 5 was applied, the KeyWord Plus co-occurrence network comprised 417 keywords. In the network, the links between the nodes indicate that keywords were frequently associated within the same publications. Three major clusters are evident, centered on the keywords "ameloblastoma"(total link strength of 1,015), "odontogenic tumors" (743) and "odontogenic keratocyst" (546).

Fig. 6. WordCloud illustration

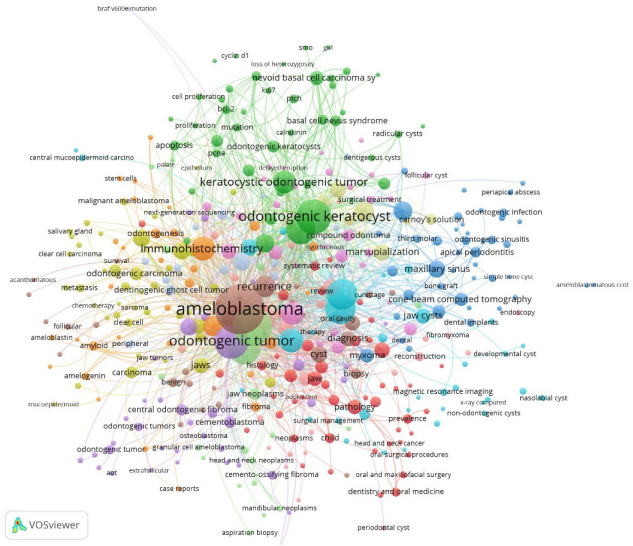

Fig. 7. Keyword co-occurrence network

Analysis of affiliations

A total of 34 institutions that published more than 20 documents and received at least 20 citations were analyzed using VOSviewer (Fig. 8). The 4 institutions with the highest total link strength were the University of Minas Gerais, Belo Horizonte, Brazil (total link strength of 25,142), the University of São Paulo, Brazil (17,824), the State University of Campinas, Brazil (16,168), and Peking University, China (11,205).

Analysis of co-citation

Figure 9 illustrates the co-citation network of the publications related to OLs. In this network, the nodes represent the cited references, and the links between the nodes indicate co-citation relationships. Co-citation analysis identified a total of 1,270 nodes and 11,617 links. The top 10 articles in terms of citation count, co-citation frequency and citation burst strength are summarized in Table 2. A study by Reichart et al.[15] was the most cited publication, receiving 816 citations. An article by Wright and Vered[25] had the largest node radius, indicating the highest co-citation frequency, with 148 co-citations. A study by Slootweg and El-Naggar[18] exhibited the strongest citation burst (strength = 89.15), with a duration of 5 years.

Analysis of clusters

When the studies were automatically clustered, several major thematic groups emerged, including cluster #0 "odontogenic keratocyst", cluster #1 "odontogenic myxoma", cluster #2 "calcifying epithelium", cluster #3

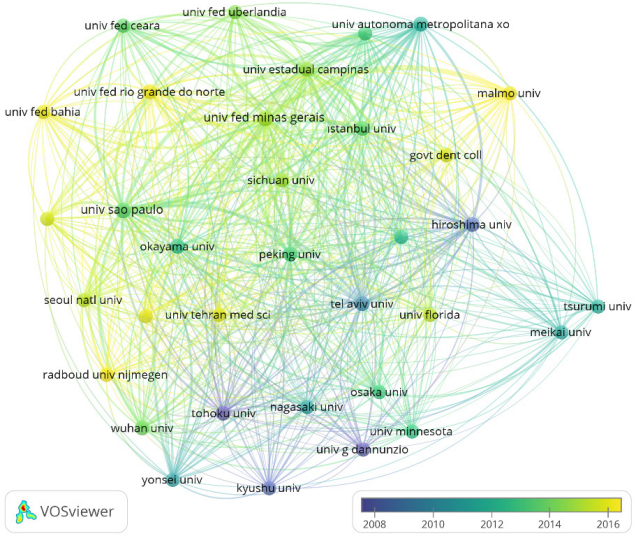

Fig. 8. Cooperation map of the most productive institutions

"calcifying odontogenic lesion", cluster #4 "ameloblastoma", and cluster #5 "apical periodontal cyst". The timeline visualization of the network, which incorporates publication characteristics such as cluster distribution and citation bursts, is presented in Fig. 10.

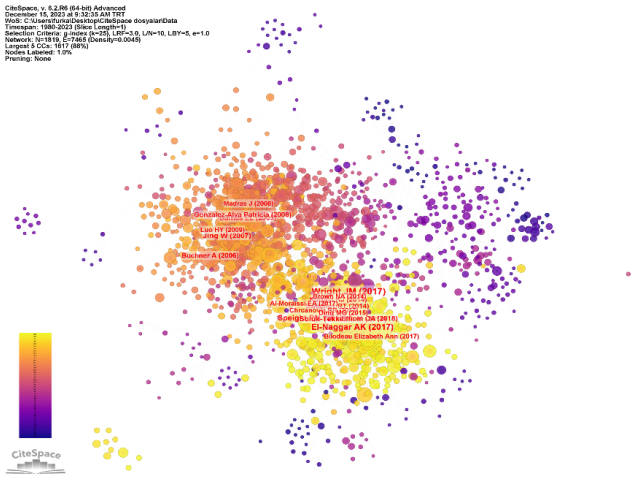

Fig. 9. Co-citation map of references from publications on odontogenic lesions (OLs)

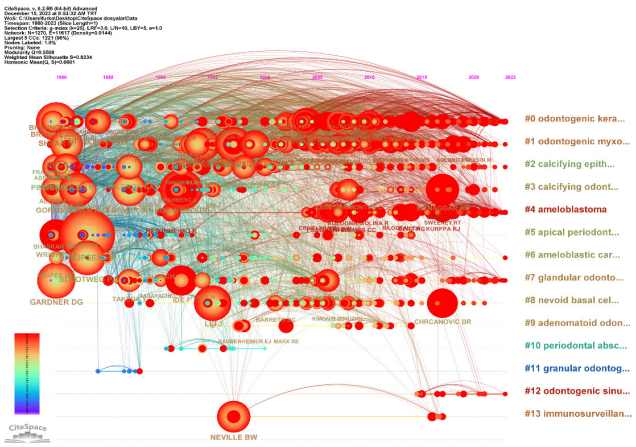

Fig. 10. Timeline view of the burst words from publications on odontogenic lesions (OLs)

Analysis of topic trends

Figure 11 illustrates the temporal trends of OL research topics over the period 1994–2021. Topic trends were identified based on term frequency, with larger point sizes indicating higher frequencies. In the early 2000s, research predominantly focused on terms such as "gene expression" (25), "keratin" (14) and "monoclonal antibodies" (7). In contrast, more recent studies have increasingly emphasized topics such as "risk" (13), "cone-beam computed tomography" (23) and "prevalence" (36).

Discussion

The Leiden Manifesto is widely regarded as an important guide in bibliometrics.[36] Comprising 10 principles, it aims to discourage the use of persuasive or overly conclusive statements in bibliometric analyses, and instead promotes the transparency and contextualized interpretation of data.[36] Bibliometric analysis has emerged as a widely used approach for evaluating the impact and productivity of articles, journals, authors, institutions, and countries, as well as for mapping collaborative networks. By systematically analyzing the scientific literature within a specific field, bibliometric methods enable the identification of research trends, the evolution of topics over time and potential future research directions.[14]

In dentistry, evaluating lesions and critically reviewing the associated scientific literature are essential for informed clinical decision making and improved patient care.[37] Accordingly, the present study provides a comprehensive analysis of global publication trends related to OLs and tracks the development of OL research over the past 4 decades. Visualization techniques and bibliometric mapping were employed to enhance the interpretability of the findings. The results offer insights into recent advances in the field, including patterns of global research collaboration, trends in clinical research and the emerging areas of interest.

Table 2. Top 10 articles with the highest metric values

Top 10 most cited articles		Top 10 most co-cited articles		Top 10 articles with the greatest citation burst strength		
Count	Reference	Count	Reference	Year range	Strength	Reference
816	Reichart et al.[15]	148	Wright and Vered[25]	2018–2023	89.15	Slootweg and El-Naggar[18]
627	Shear and Speight[16]	97	Slootweg and El-Naggar[18]	2018–2023	73.5	Wright and Vered[25]
549	Gardner[17]	71	Speight et al.[26]	2018–2023	72.82	Chrcanovic et al.[32]
479	Slootweg and El-Naggar[18]	52	Soluk-Tekkesin and Wright[7]	1987–1993	56.92	Kramer et al.[23]
456	Gorlin[19]	50	Jing et al.[27]	1980–2001	50.08	Pindborg et al.[33]
427	Buchner et al.[20]	40	Buchner et al.[20]	1987–1991	35.49	Waldron and El-Mofty[34]
415	Lu et al.[21]	39	Effiom et al.[28]	1980–1999	35.1	Gorlin[19]
410	Regezi[22]	38	Barnes et al.[29]	2017–2023	33.29	Kurppa et al.[31]
400	Kramer et al.[23]	38	Sweeney et al.[30]	2019–2023	32.73	Speight et al.[26]
376	Reichart[24]	37	Kurppa et al.[31]	1980–2000	32.63	Shear and Pindborg[35]

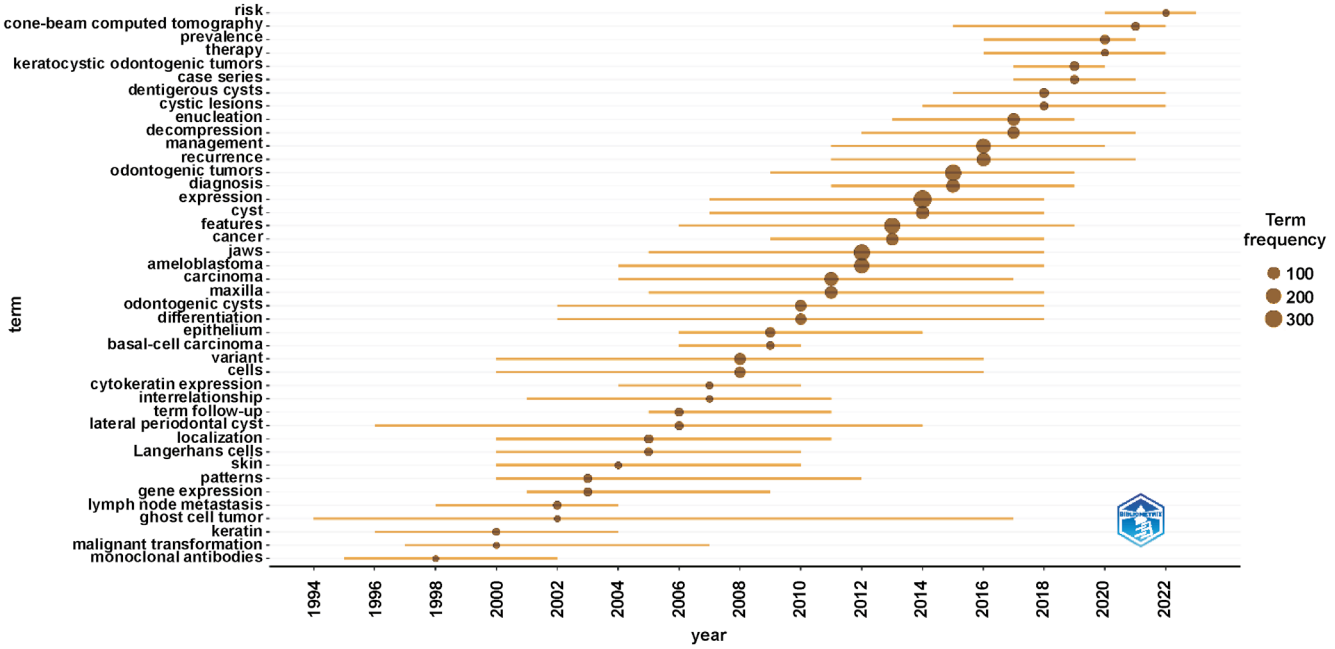

Fig. 11. Topic trends in publications on odontogenic lesions (OLs) by year

The number of publications on OLs in dental journals increased markedly during the 2010s, reaching a peak in 2022, followed by a decline in 2023. Given that the preparation, submission and peer-review processes of academic publications require substantial time, the COVID-19 pandemic may have contributed to the observed decrease in publications from 2022 onward. This influence may reflect disruptions in research priorities, workflows and productivity among investigators studying OLs during the pandemic period (2019–2021).[38] From the patient perspective, the postponement of elective dental treatment procedures and the disruption of routine check-ups during the same period likely resulted in fewer diagnosed and treated OL cases. Łazarz-Półkoszek et al. reported that, despite efforts to establish optimal sanitary conditions, patients remained anxious about attending dental appointments during the COVID-19 pandemic.[39] Reduced patient motivation and attendance may have further delayed the diagnosis and management of OLs, particularly in asymptomatic cases. We anticipate that the normalization of dental care services in the post-pandemic period will lead to a relative increase in the detection and reporting of OL cases, which is expected to be reflected in future publications. Moreover, the observed AGR of 7.01% in the literature supports the view that OLs will remain an important and increasingly discussed topic in the coming years (Fig. 1).

India had the highest number of publications (670) but ranked 6th in total citations (2,794). In contrast, the USA ranked 2nd in the publication count (609), yet achieved the highest total number of citations (12,169). Consistent with numerous bibliometric studies in oral and maxillofacial surgery, the USA continues to lead in both citation impact and research output, a trend that is expected to persist in the future (Fig. 3).[14,40,41]

International collaboration varied considerably across countries and authors. Although India produced the largest number of publications, it collaborated with only 31 countries. By comparison, Germany, despite publishing substantially fewer articles (122), demonstrated broader international engagement, collaborating with 38 countries. One possible explanation for the relatively lower citation impact of Indian publications is their frequent appearance in locally indexed journals, which may limit international visibility and collaboration. Strengthening cross-border research partnerships, particularly for high-output countries such as India and Japan, could enhance the research quality, clinical applicability and global impact of future OL studies (Fig. 4).

Cooperation analysis was employed to evaluate collaborative interactions among institutions. In the network visualization, the node size represents the number of publications produced by each institution, while the link thickness reflects the intensity of collaboration. Overall, the level of cooperation among countries and institutions was sufficient to sustain a robust scientific network. Notably, several Brazilian institutions are highlighted in yellow and light green, indicating active and ongoing contributions to the OL literature. Given that Brazil ranks 3rd in national research productivity (536) and hosts multiple institutions with strong engagement in OL research, it may be inferred that Brazil has the potential to emerge as a leading country in this field in the coming years.

The WordCloud is a useful visualization tool for identifying key terms that define a research topic and for guiding future studies. In this type of visualization, the font

size, color coding and the spatial arrangement of words provide insights into keyword frequency, relative importance and associations with other topics. In keyword co-occurrence analysis, larger nodes or words typically indicate higher dominance of a keyword, smaller distances between 2 nodes suggest stronger relationships and thicker links reflect more frequent co-occurrence of 2 keywords.

A wide variety of keywords were identified, ranging from lesion types, such as "keratocysts", "ameloblastomas" and "carcinomas", to surgical procedures, including "endoscopy" and "aspiration", as well as molecular and genetic factors, such as cell cycle-related proteins ("Bcl-2" (B-cell lymphoma 2), "Cyclin D1") and cancer-associated mutant genes (e.g., "*BRAF V600E*"). A particularly prominent keyword in recent years is "next-generation sequencing". Studies utilizing this technology to detect pathogenic variants in ameloblastoma, particularly somatic mutations such as *FGFR2* and *SMO*, may serve as important references for future research.[42] Three keywords – "odontogenic sinusitis", "maxillary sinusitis" and "cone-beam computed tomography" – were highlighted due to their recent rise in frequency. The results indicate that researchers are now paying more attention to evaluating OLs in the maxillary sinus with cone-beam computed tomography (CBCT), which is widely regarded as the gold standard for sinus imaging.[43]

The primary objective of co-occurrence analysis is to map the conceptual structure of a research field by examining how words co-occur within a bibliographic collection. Keywords play a critical role in disseminating research to a broader audience and in influencing how findings are recognized within the scientific community.[44] However, overreliance on generic keywords can weaken research visibility, and potentially lead to the misinterpretation of results.[45] Literature reviews recommend a balanced approach that incorporates both generic and specific keywords to prevent overlooking relevant evidence while minimizing misleading conclusions.[45] In the present study, the high density of generic terms such as "mandible", "maxilla" or "jaw" may overshadow more specific keywords that could guide future research. The authors therefore suggest limiting the excessive use of generic terms to enhance clarity and relevance in bibliometric analyses.

Co-citation coupling is a technique used to assess subject similarity between 2 documents. When both documents appear in the reference list of a third document, they are considered co-cited, indicating a relational link between them. The strength of this relationship increases as the number of shared citations across different sources grows. Co-citation frequency reflects how often 2 documents are cited together, and serves as an indicator of their conceptual or thematic similarity.[46]

In this study, the most cited, most co-cited and strongest citation-burst articles related to OLs were evaluated. When analyzing Table 2, the publications with the greatest citation burst strength were the article by Slootweg and El-Naggar entitled "World Health Organization 4th edition of Head and Neck Tumour Classification: Insight into the consequential modifications"[18] and the article by Wright and Vered entitled "Update from the 4th edition of the World Health Organization Classification of Head and Neck Tumours: Odontogenic and maxillofacial bone tumors".[25] In addition to being the 2 studies with the strongest citation burst in the last 5 years, they were also the most co-cited publications. This can be explained by the fact that these 2 publications reviewed the 4th edition of the World Health Organization (WHO) classification of head and neck tumors published in 2017. The significance of employing a precise and up-to-date classification system is particularly evident in situations where differential diagnosis is challenging. In this regard, Gupta et al. demonstrated that differences in the reported frequency distribution of gingival lesions across 2 distinct classification systems were attributable to heterogeneous terminology rather than actual geographical variations.[47]

As shown in Fig. 10, the publications exhibiting citation bursts (the red rings surrounding larger circular areas) first appeared in the early 1980s, with the number of such circular areas continuing to increase over time. This sustained pattern over the past 4 decades suggests that research on OLs has remained of ongoing scientific interest. The analysis of the timeline view and the clustering of topics further reveals a shift in the thematic focus, with earlier studies commonly addressing topics such as apical periodontal cysts, ameloblastic carcinoma and glandular odontogenic cysts, whereas more recent research has increasingly emphasized themes such as ameloblastoma and maxillary sinus involvement. Publications describing a specific tumor, e.g. those by Gorlin[19] and Pindborg et al.,[33] experienced a citation burst between 1980 and 2000. For example, the calcifying odontogenic cyst (Gorlin's cyst) was first described by Gorlin in 1980 as an intraosseous, solid, non-neoplastic cystic lesion.[19] In the 2017 WHO classification, Slootweg and El-Naggar categorized this entity under the category "Developmental Odontogenic Cysts", and this publication subsequently demonstrated a notable citation burst.[18] Given the strength of this citation burst, this work is expected to gain increasing prominence in the coming years and to serve as a key reference for future studies on calcifying odontogenic cysts.

The trending topics related to OLs were analyzed as well. As expected, the most frequently occurring terms were "tumors" (325) and "lesions" (275). In the early 2000s, research predominantly focused on pathological and molecular concepts, with a frequent use of terms such as "gene expression" (25), "keratin" (14) and "monoclonal antibodies" (7). In contrast, more recent years have seen increased emphasis on diagnostic and epidemiological concepts, reflected by the prominence of terms

such as "risk" (13), "cone-beam computed tomography" (23) and "prevalence" (36). This shift highlights a transition in OL research from primarily pathological characterization toward diagnostic evaluation and population-based assessment. Cone-beam computed tomography enables the three-dimensional (3D) evaluation of the maxillofacial region while delivering a relatively low radiation dose.[48] Introduced into dental practice in the late 1990s, this technology has substantially advanced diagnostic and treatment planning capabilities in dentistry.[49] Shweel et al. reported that CBCT was the most appropriate radiological modality for the preoperative assessment of odontogenic tumors.[50] These advantages likely explain why CBCT has emerged as a prominent topic in the OL literature.

The high frequency of keywords such as "prevalence" and "risk" further indicates that assessing the epidemiology of OLs and evaluating their potential clinical risks are current priorities in the literature. It is therefore anticipated that research focusing on the prevalence, treatment, risk assessment, and imaging of OLs will continue to attract significant scholarly attention in the coming years.

Limitations

This study has several limitations. First, the data source was restricted to the literature indexed in the Web of Science (WoS) database; therefore, relevant publications from other databases may not have been included. Second, no formal assessment of bias was conducted when evaluating the included publications, and the study findings were presented without the critical appraisal of methodological quality. These limitations should be considered when interpreting the results.

Conclusions

In the near future, studies focusing on the histological and clinical characteristics of various odontogenic cysts and tumors are expected to remain highly relevant, while research on prevalence, diagnostic approaches, follow-up protocols, and management strategies is likely to gain parallel importance. Conducting similar bibliometric analyses in the future will enable evidence-based monitoring of research progress, and provide valuable insights into the emerging trends and future directions in the literature.

Ethics approval and consent to participate

This study was conducted in accordance with the principles set forth in the Leiden Manifesto. Bibliometric analysis is exempt from the institutional ethics committee review, as it relies solely on publicly available electronic sources and does not involve the generation of novel data or the use of private patient information.

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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Review

Laser therapy for the management of oral mucositis: An umbrella review – official recommendations of the Polish Society for Laser Dentistry

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Abstract

Oral mucositis (OM) is a common and debilitating side effect of cancer therapy that impairs nutrition, increases infection risk, and often disrupts oncologic treatment. Photobiomodulation therapy (PBMT) has emerged as an effective, non-invasive method for OM prevention and management.

This umbrella review was conducted in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 guidelines and the Joanna Briggs Institute (JBI) methodology. A comprehensive search of PubMed®/MEDLINE, Embase, Scopus, and Cochrane Library identified systematic reviews and meta-analyses on laser therapy for OM published through July 2025. The data extraction process centered on clinical outcomes, laser parameters and safety.

Twenty-two reviews met the inclusion criteria. Photobiomodulation therapy significantly reduced the incidence of OM, disease severity, pain, and healing time across adult and pediatric populations. Preventive PBMT decreased the risk of severe OM (grade 3–4) by 40–80%, while therapeutic PBMT shortened ulcer duration by 4–7 days. The combination of PBMT and photodynamic therapy (PDT) enhanced mucosal healing and alleviated pain. Optimal outcomes were achieved when wavelengths of 630–670 nm (intraoral) and 780–850 nm (extraoral) were used, with fluences of 2–6 J/cm^2. No serious adverse events were reported.

Photobiomodulation therapy demonstrates strong efficacy and safety in the management of OM, improving quality of life and treatment continuity in oncology patients. The Polish Society for Laser Dentistry (PTSL) endorses PBMT as a standard supportive care modality, particularly in the context of hematopoietic stem cell transplantation (HSCT) and head and neck chemoradiation. Protocol adherence and parameter standardization are essential to ensure the reproducibility and clinical effectiveness of research findings.

Keywords: low-level laser therapy, head and neck cancer, hematopoietic stem cell transplantation, cancer therapy, photobiomodulation

Highlights

- Photobiomodulation therapy significantly reduces the incidence, severity and duration of cancer therapy-induced oral mucositis while improving pain control and overall patient quality of life.
- Both preventive and therapeutic protocols show excellent safety, allowing chemotherapy, radiotherapy, or stem cell transplantation to proceed without interruption or treatment delays.
- International guidelines (MASCC/ISOO, WALT, NICE) as well as the Polish Society for Laser Dentistry recommend photobiomodulation as standard supportive care for oncology patients at high risk of oral mucositis.
- The use of standardized laser parameters and thorough documentation supports reproducible outcomes and optimal clinical effectiveness in both adult and pediatric populations.

Introduction

Rationale

Oral mucositis (OM) represents one of the most debilitating and clinically significant complications of cancer therapy, affecting the oral and gastrointestinal mucosa and carrying potentially life-threatening consequences.[1–4] This inflammatory condition, characterized by erythema, edema and ulceration of the mucous membranes lining the oral cavity, occurs as a direct result of the cytotoxic effects of chemotherapy (CT) and radiotherapy (RT) on rapidly dividing epithelial cells.[5] The clinical manifestations of OM range from mild discomfort and erythema to severe confluent ulcerations that can prevent oral intake, necessitate narcotic analgesics and require parenteral nutrition.[6–8] The epidemiological burden of OM is substantial and varies significantly based on treatment modality and patient characteristics. Mucositis contributes to prolonged hospitalization, higher infection rates, and delays or reductions in CT. Excluding high-risk cases such as hematopoietic stem cell transplantation (HSCT) and RT, the incidence of this condition ranges from 5% to 15%. Up to 40% of patients receiving 5-fluorouracil (5-FU), with or without leucovorin, develop OM, with 10–15% of these cases being classified as severe. Irinotecan has been associated with severe gastrointestinal mucositis in over 20% of patients. In bone marrow transplant recipients, OM is reported in 75–85% of cases and is frequently the most severe side effect of the treatment. Melphalan-based regimens are particularly associated with high rates of the condition.[9–13] The clinical and economic impact of OM extends far beyond the immediate discomfort experienced by patients. This condition has a significant influence on quality of life, disrupting normal oral functions such as eating, swallowing and speaking. Furthermore, it has been identified as a portal for potentially life-threatening infections.[14] The healthcare burden is considerable, with OM-related complications contributing to increased hospitalization rates, extended length of stay, and substantial healthcare costs.[15] Despite the advances in supportive care and increased understanding of the pathophysiology of mucositis, effective prevention and management strategies remain limited.[16–19] The condition involves a complex, multi-phase pathological process including initial tissue injury, inflammatory amplification, ulceration with bacterial colonization, and eventual healing phases. This complexity, in conjunction with the heterogeneity of cancer treatments and patient populations, has rendered the development of universally effective interventions challenging.

Oral mucositis grading scale

The World Health Organization (WHO) scale is a standardized tool used to assess the severity of OM by combining both subjective symptoms and objective clinical findings.[19] This grading system ranges from 0 to 4, with grade 0 indicating no signs of OM. Grade 1 is characterized by erythema and soreness without ulceration. Patients with grade 2 OM typically present with ulceration, yet are still able to consume solid foods. Grade 3 involves ulcers severe enough to require a liquid diet due to pain or difficulty chewing and swallowing. Grade 4 is the most severe stage, where extensive ulceration makes oral alimentation impossible. This scale is widely used in clinical and research settings for the evaluation of treatment effects and the guidance of patient care.[19]

Objectives

The aim of this umbrella review was to systematically collect, evaluate and synthesize the highest level of existing evidence from systematic reviews and meta-analyses on the use of laser therapy for the prevention and management of OM in cancer patients. Specifically, the review sought to assess the clinical effectiveness of various laser modalities in reducing the incidence, severity, duration, and pain associated with mucositis, and to evaluate the consistency of findings across different patient populations and treatment protocols. By consolidating current evidence, the study intends to inform clinical guidelines and support evidence-based recommendations by the Polish Society for Laser Dentistry (PTSL).

Material and methods

This study was carried out in accordance with the Joanna Briggs Institute (JBI) framework for umbrella reviews and was registered with PROSPERO (ID: CRD420251119913).[20]

PICO question

The following PICO question was formulated: In patients experiencing or at risk of OM (Population), does laser therapy (Intervention), as compared to standard care or no laser treatment (Comparison), lead to improved clinical outcomes such as reduced severity, duration or pain (Outcome)?[21]

Search strategy

This umbrella review was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 guidelines to ensure transparency and methodological rigor.[22] A systematic and comprehensive search of several major electronic databases, including PubMed®/MEDLINE, Embase, Scopus, and Cochrane Library, was conducted in July 2025 to identify systematic reviews and meta-analyses evaluating the use of laser therapy in the prevention or management of OM. Three independent reviewers performed the literature search using a carefully constructed combination of Medical Subject Headings (MeSH) and keywords related to OM and laser treatment. Studies published in English up to July 1, 2025, were included. The two-phase screening process involved an initial screening of titles and abstracts, followed by a full-text assessment by 3 independent reviewers using clearly defined inclusion and exclusion criteria. In order to ensure that the work is complete, the reference lists of all included reviews were examined manually to find additional relevant studies. The search strategies aimed to identify systematic reviews and meta-analyses that examined the effectiveness of laser-based interventions in the prevention and management of OM. The queries targeted literature published between 2020 and 2025, drawn from major biomedical databases (Table 1).

Study selection

The study selection process began with an initial review of titles and abstracts, guided by well-defined eligibility criteria that were tailored to the research objectives. Disagreements between the reviewers at this stage were resolved through collaborative discussion. This umbrella review targeted systematic reviews and meta-analyses that investigated the use of laser-based therapies and related approaches for the management or prevention of OM. Only studies assessing clinically relevant outcomes such as symptom severity, duration, pain level, or mucosal healing were considered for inclusion. To ensure data reliability, the inclusion criteria were limited to peer-reviewed reviews that employed clear methodology, provided comparison groups, and reported quantifiable health outcomes. Reviews were excluded in the absence of peer review, the presence of opinion-based content (e.g., editorials or narrative summaries), or if they were published only as conference abstracts or unpublished theses. Articles not written in English, which lack sufficient detail on intervention protocols, and which do not focus directly on laser interventions for OM, were also omitted. Studies that failed to differentiate between laser therapy or lacked clinical relevance were excluded from the analysis, as were duplicates and secondary reports from the same dataset, unless they offered new findings.

The PRISMA flow diagram, illustrating the study selection process for the systematic review, is presented in Fig. 1. The database search yielded a total of 264 records: 50 from PubMed®/MEDLINE; 151 from Embase; 59 from Scopus; and 4 from the Cochrane Library. An additional 2 records were identified through citation search. Prior to the screening process, 57 duplicate records were removed. Of the remaining 207 papers, 184 were excluded,

Table 1. Search strategy used in the study

Source	Search syntax	Filters	*n*
PubMed®/MEDLINE	("oral mucositis"[MeSH Terms] OR "oral mucositis"[Title/Abstract] OR "mucositis"[Title/Abstract] OR "stomatitis"[Title/Abstract]) AND ("laser therapy"[MeSH Terms] OR "low-level light therapy"[MeSH Terms] OR "photobiomodulation therapy"[Title/Abstract] OR "low-level laser therapy"[Title/Abstract] OR "low level laser"[Title/Abstract] OR "LLLT"[Title/Abstract] OR "PBMT"[Title/Abstract] OR "laser treatment"[Title/Abstract] OR "laser"[Title/Abstract])	systematic review; 2020–2025	50
Embase	('oral mucositis'/exp OR 'oral mucositis':ti,ab OR 'mucositis':ti,ab OR 'stomatitis':ti,ab) AND ('laser therapy'/exp OR 'low level laser therapy'/exp OR 'photobiomodulation therapy':ti,ab OR 'low-level laser therapy':ti,ab OR 'low level laser':ti,ab OR 'LLLT':ti,ab OR 'PBMT':ti,ab OR 'laser treatment':ti,ab OR 'laser':ti,ab)	review; 2020–2025	151
Scopus	(TITLE-ABS-KEY("oral mucositis" OR "mucositis" OR "stomatitis")) AND (TITLE-ABS-KEY("laser therapy" OR "low-level laser therapy" OR "low level laser" OR "photobiomodulation therapy" OR "low-level light therapy" OR "laser treatment" OR "laser" OR "LLLT" OR "PBMT"))	systematic review; 2020–2025	59
Cochrane Library	("oral mucositis" OR "mucositis" OR "stomatitis") AND ("laser therapy" OR "low-level laser therapy" OR "low level laser" OR "photobiomodulation therapy" OR "low-level light therapy" OR "laser treatment" OR "laser-assisted" OR "laser" OR "lasers" OR "LLLT" OR "PBMT")	review; 2020–2025	4

MeSH – medical subject headings; ti – title; ab – abstract; exp – explosion (Emtree terms).

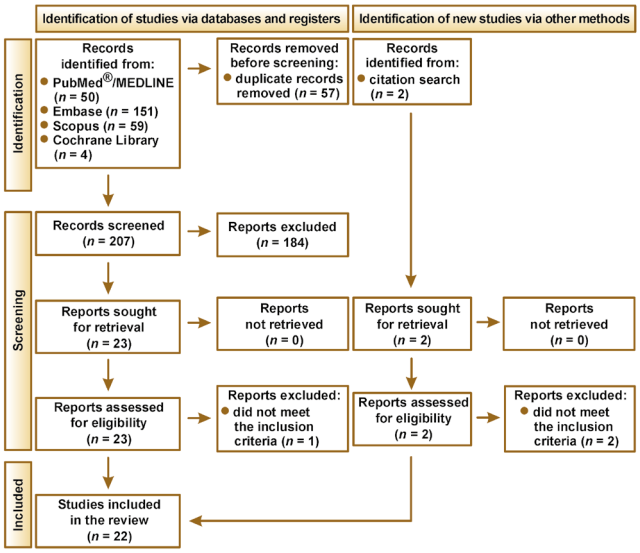

Fig. 1. Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 flow diagram of the study

and 23 reports were sought for retrieval. A total of 23 reports were successfully retrieved and assessed for eligibility. One report was excluded as it did not meet the criteria for being a systematic review or a meta-analysis. Ultimately, 22 studies were included in the review.

Data extraction

Once the final set of eligible reviews was established, 3 reviewers independently extracted data using a standardized protocol designed to ensure consistency and minimize bias. Key information collected from each included review comprised the first author, the year of publication, the review type (systematic review or meta-analysis), the clinical context, and characteristics of the studied population. Particular attention was given to the details regarding the photobiomodulation therapy (PBMT)/low-level laser therapy (LLLT) or laser therapy protocols, encompassing the type of laser or light source used, wavelength, power output, energy density, application technique, and treatment duration. Where available, information on the frequency of treatment, its timing relative to CT or RT, and whether laser therapy was used preventively or therapeutically was also extracted. Data on primary and secondary outcomes, such as mucositis severity, duration, pain relief, and impact on quality of life, was recorded to facilitate a comparative analysis across studies and to evaluate the consistency and clinical relevance of reported effects.

Assessment of the risk of bias and study quality

The methodological quality of each included study was assessed independently by 3 reviewers using a customized risk-of-bias assessment tool adapted for the evaluation of systematic reviews on therapeutic interventions. The tool covered 9 domains designed to capture both reporting quality and internal validity. The assessment criteria encompassed the following:

– clear identification and description of the laser modality used, including treatment parameters where applicable;
– defined intervention protocols, such as treatment frequency and adjunctive care;
– specification of relevant clinical outcomes;
– inclusion of appropriate comparator groups;
– transparent criteria for study inclusion, including population characteristics;
– assessment of bias control measures, including blinding where feasible;
– appropriateness and transparency of statistical methods used;
– full disclosure and clarity in the reporting of outcomes, including adverse events and follow-up data;
– reporting of funding sources and potential conflicts of interest.

Each domain was scored using a binary system (1 for criterion met, 0 for unmet), yielding a total score between 0 and 9. The reviews were classified as having low (7–9 points), moderate (4–6 points) or high (0–3 points) risk of bias. Disagreements in scoring were resolved through reviewer discussion, with consultation from a fourth reviewer in cases of unresolved conflict. The quality appraisal process was conducted in accordance with the best-practice guidance from the Cochrane Handbook for Systematic Reviews of Interventions.[23]

Results

Results of the risk of bias and quality assessment

The initial screening of titles and abstracts was conducted independently by 3 reviewers to promote objectivity and reduce the risk of selection bias. Agreement among reviewers was evaluated using Cohen's kappa coefficient to quantify the level of consistency across assessments. When discrepancies in the study inclusion arose, they were resolved through structured consensus meetings to ensure transparency and consistency in the selection process. This multi-reviewer approach was adopted to enhance the methodological rigor of the umbrella review and to ensure that only systematic reviews and meta-analyses, specifically those evaluating laser therapy for OM, were included.[24]

As summarized in Table 2, all but one study were judged to be at low risk of bias. Importantly, no studies were excluded on the basis of their risk-of-bias rating alone.

Characteristics of the included reviews

Multiple studies have demonstrated the efficacy of laser-based interventions, particularly PBMT, in reducing the

Table 2. Assessment of the risk of bias in the included studies

Study	Score (0–9)	Risk of bias
Alqahtani and Khan 2022[25]	6	moderate
Andriakopoulou et al. 2024[26]	7	low
Braguês et al. 2024[27]	7	low
Calarga et al. 2024[28]	9	low
Campos et al. 2020[29]	8	low
Cronshaw et al. 2020[30]	8	low
Cronshaw et al. 2020[31]	8	low
Cruz et al. 2023[32]	9	low
de Oliveira et al. 2021[33]	8	low
de Sales et al. 2025[34]	8	low
Dipalma et al. 2024[35]	7	low
Franco et al. 2023[36]	8	low
Joseph et al. 2024[37]	8	low
Joseph et al. 2025[38]	7	low
Khalil et al. 2024[39]	8	low
Lai et al. 2021[40]	9	low
Parra-Rojas et al. 2025[41]	8	low
Peng et al. 2020[42]	9	low
Potrich et al. 2023[43]	8	low
Redman et al. 2022[44]	7	low
Sánchez-Martos et al. 2023[45]	8	low
Shen et al. 2024[46]	7	low

severity and pain associated with OM in patients with head and neck cancer, especially in pediatric populations.[25–28] Alqahtani and Khan elucidated that a combination of oral care, glutamine, vitamin E, biological agents, and laser therapy effectively alleviated the symptoms of OM in children, with PBMT exhibiting a consistent reduction in pain and severity of the condition.[25,26] Braguês et al. noted the preventive effects of interventions such as PBMT, palifermin, honey, and zinc, however, they emphasized the absence of standardized protocols.[27] A study by Calarga et al. confirmed the safety and effectiveness of PBMT, although preventive outcomes varied and the absence of established protocols underscored the need for standardization.[28] Campos et al. found that PBMT not only reduced OM severity but was also cost-effective in patients with head and neck cancer.[29] Cronshaw et al. emphasized that optical parameters, particularly spot size and energy delivery, critically influence PBMT outcomes.[30,31] The authors recommended the implementation of individualized dosing based on tissue depth and the patient.[30,31] Cruz et al. reported that PBMT significantly reduced OM severity, though the heterogeneity of outcomes precluded a meta-analysis on pain or lesion duration.[32] A study by de Oliveira et al. found that combining photodynamic therapy (PDT) with PBMT led to a significant acceleration in mucosal healing compared to PBMT alone.[33] De Sales et al. linked PBMT to a reduction in OM incidence and inflammation through cytokine modulation and enhanced antioxidant activity.[34] Dipalma et al. echoed these findings, showing that PBMT has an impact on cytokines and keratinocyte differentiation.[35] This suggests that its mechanism is driven by the modulation of inflammation and oxidative stress.[35] Franco et al. concluded that laser therapy significantly reduces OM severity, especially in patients undergoing transplantation or chemoradiation.[36] Joseph et al. showed that PDT combined with PBMT offered greater pain and symptom relief in comparison to PBMT alone, with meta-analysis supporting superior efficacy.[37] Another study by Joseph et al. explored the potential of light-emitting diode (LED)-based therapy, which showed promise in symptom control despite limited evidence and variability across studies.[38] Khalil et al. found that PBMT using 660-nm aluminium gallium indium phosphide (InGaAlP) lasers led to a consistent reduction in OM severity, with IL-6 levels demonstrating the strongest correlation with the intensity of mucositis.[39] Lai et al. reported that cryotherapy combined with PBMT was more effective than either intervention alone in reducing severe OM; however, no significant differences were observed for moderate OM.[40] Parra-Rojas et al. reported that prophylactic PBMT effectively prevented OM, with red light used intraorally and infrared extraorally, but emphasized the need for protocol standardization.[41] Peng et al. confirmed the enhanced effect of cryotherapy + PBMT, both outperforming usual care, especially in severe OM cases.[42] Potrich et al. also supported this synergy and emphasized the efficacy of both modalities individually.[43] Redman et al. found that while PBMT may benefit children with CT-induced OM, results were inconsistent and further trials are needed.[44] Sánchez-Martos et al. highlighted the ability of PBMT to decrease severe OM incidence and duration, reduce pain, and improve quality of life across various assessment tools.[45] Finally, Shen et al. confirmed the broad efficacy of PBMT, particularly in pediatric patients, and reinforced its safety profile, emphasizing the importance of standardized treatment protocols.[46] Tables 3 and 4 summarize this data.

Table 3. Summary of interventions and outcomes for the management of oral mucositis (OM) in cancer patients based on the included studies

Study	Type	Analyzed studies, *n*	Interventions	Databases	Publication year range
Alqahtani and Khan 2022[25]	SR	15	oral care, laser therapy, glutamine, SCPR, vitamin E	Embase, PubMed®, ScienceDirect, Cochrane Library, hand search	2005–2021
Andriakopoulou et al. 2024[26]	SR	34	honey, PBMT	MEDLINE (via PubMed®), Scopus	January 2000–March 2023
Braguês et al. 2024[27]	SR	39	PBMT, palifermin, honey, zinc	PubMed®/MEDLINE, NICE, ICTRP, Embase (MEDLINE excluded), Scopus, Web of Science	last 20 years up to March 2024
Calarga et al. 2024[28]	SR	20	PBMT	PubMed®, Embase, Cochrane Library, Google Scholar (gray literature)	not specified, literature search performed on May 10, 2023
Campos et al. 2020[29]	M	13	PBMT	PubMed®, Web of Science, MEDLINE	2007–2018
Cronshaw et al. 2020[30]	M	38	PBMT	PubMed®, Cochrane Library, Google Scholar	last 10 years, search performed from April 8, 2020 to June 15, 2020
Cronshaw et al. 2020[31]	SR	29	PBMT	PubMed®, Google Scholar, Cochrane Library, manual search	1995–2019
Cruz et al. 2023[32]	M	6	PBMT	PubMed®, Scopus, Cochrane Library, Web of Science	2013–2023
de Oliveira et al. 2021[33]	M	5	PDT	PubMed®, Scopus, Web of Science	2000–2020
de Sales et al. 2025[34]	SR	7	PBMT	PubMed®/MEDLINE, Cochrane Library, Web of Science, LILACS, ClinicalTrials.gov	up to April 2023
Dipalma et al. 2024[35]	SR	11	PBMT	PubMed®, Cochrane Library, Embase, Scopus, Web of Science	2010–2023
Franco et al. 2023[36]	M	3	PBMT + diode laser	PubMed®, Scopus, Web of Science	not specified, search performed on May 3, 2023
Joseph et al. 2024[37]	SR	7	LED-based PBMT	PubMed®/MEDLINE, Scopus, Web of Science	January 2000–May 2024
Joseph et al. 2025[38]	M	5	PDT, PBMT	major databases (not fully listed in the abstract)	not specified
Khalil et al. 2024[39]	SR	4	PBMT (InGaAlP laser)	Web of Science, Embase, ScienceDirect, PubMed®, Cochrane Library, Scopus	up to February 2024
Lai et al. 2021[40]	M	26	PBMT, cryotherapy, or both	MEDLINE, Embase, CENTRAL, PEDro	up to 2020
Parra-Rojas et al. 2025[41]	SR	13	preventive PBMT	PubMed®, Scopus, Web of Science, Cochrane Library	January 2010–May 2024
Peng et al. 2020[42]	M	30	prophylactic and therapeutic PBMT	PubMed®, Embase, CENTRAL, Web of Science	up to October 2019
Potrich et al. 2023[43]	SR	7	PBMT	Cochrane Library, Embase, PubMed®, Scopus, Web of Science	not specified
Redman et al. 2022[44]	M	14	PBMT	multiple databases and gray literature (not listed)	not specified
Sánchez-Martos et al. 2023[45]	SR	10	PBMT	PubMed®, Scopus, Cochrane Library	not specified, search performed between November 2021 and February 2022
Shen et al. 2024[46]	M	14	PBMT	PubMed®, Embase, Cochrane Library, LILACS, Web of Science	January 2000–October 2023

CENTRAL – Cochrane Central Register of Controlled Trials; ICTRP – International Clinical Trials Registry Platform; InGaAlP – aluminium gallium indium phosphide; LED – light-emitting diode; LILACS – Latin American and Caribbean Literature on Health Sciences Database; M – meta-analysis; NICE – National Institute for Health and Care Excellence; OM – oral mucositis; PBMT – photobiomodulation therapy; PDT – photodynamic therapy; ROBINS-I – Risk Of Bias In Non-randomized Studies – of Interventions; SCPR – supersaturated calcium phosphate rinse; SR – systematic review.

Study design	Assessment of the risk of bias	Outcome
clinical and research works published in English involving children aged ≤18 years with OM	not specified	reduced pain and OM severity
randomized controlled trials	Jadad scale (qualitative synthesis) + Cochrane Risk of Bias tool (meta-analysis)	administration of honey reduced hospital stay; PBMT not effective for OM ≥grade 2
studies on pediatric patients (not all specified as randomized controlled trial) meeting inclusion criteria	not specified	preventive effects and symptom improvement
randomized controlled trials; included studies that made comparisons between PBMT protocols, with no other comparators	Cochrane Risk of Bias 2	PBMT reduced severity, duration and pain; safe intervention
randomized clinical trials	not specified	clinically effective and cost-effective intervention
randomized controlled clinical trials	modified Cochrane Risk of Bias tool	effectiveness associated with specific optical parameters
human randomized controlled clinical trials, retrospective case analyses	not specified	prophylactic PBMT provided measurable clinical benefits
randomized controlled trials	Cochrane Risk of Bias 2	reduced OM severity
randomized controlled trials	Cochrane Risk of Bias tool	effective in healing alone and in combination with PBMT
randomized controlled trials	Cochrane Risk of Bias 2	reduced OM incidence and inflammation
randomized controlled trials	Cochrane Risk of Bias 2	PBMT lowered OM incidence and pain; improved tissue regeneration
randomized controlled trials with placebo control	not specified	reduced OM severity and duration
randomized controlled trials and non-randomized clinical trials	Cochrane Risk of Bias 2 + ROBINS-I	reduced pain and OM severity; low certainty of evidence
randomized controlled trials and non-randomized intervention studies	Cochrane Risk of Bias 2 + ROBINS-I	PDT + PBMT improved healing and reduced pain compared with PBMT alone
clinical trials	several appraisal tools (not specified)	reduced OM severity and modulated salivary cytokines (notably IL-6)
randomized controlled trials	Cochrane Collaboration's tool	all interventions improved OM severity; combination therapy most effective for mild/severe OM
randomized controlled trials	Cochrane Risk of Bias 2	prophylactic PBMT reduced OM risk and lesion intensity during cancer therapy
randomized controlled trials	Cochrane Risk of Bias tool	reduced incidence and duration of severe OM; analgesic use; less severe pain
studies assessing quality of life in head and neck cancer patients undergoing PBMT	not specified	reduced OM severity and maintained quality of life
randomized controlled trials (efficacy), all study types (safety)	not explicitly stated, risk of performance bias discussed	showed potential to reduce OM severity and oral pain
clinical trials	not specified	prophylactic PBMT reduced OM severity (grades 3–4), duration and pain; improved quality of life
randomized controlled trials	GRADE + Cochrane Risk of Bias tool	lower OM incidence from week 2; reduced severe OM from week 3; decreased OM-related pain

Table 4. Extended summary of outcomes for the management of oral mucositis (OM) in cancer patients based on the included studies

Study	Main outcomes
Alqahtani and Khan 2022[25]	• Multiple interventions, including good oral care, glutamine, vitamin E, aloe vera, olive oil, and laser therapy, were shown to reduce OM severity and pain in children receiving CT or stem cell transplants. • Laser therapy, especially PBMT, was consistently effective in pediatric settings. • Glutamine reduced the incidence of OM in acute lymphoblastic leukemia from 62.5% to 4.2% compared to placebo. • Oral cryotherapy and caphosol showed little or no benefit, indicating the need for improved strategies.
Andriakopoulou et al. 2024[26]	• Several methods were found to reduce OM severity and discomfort in children receiving CT or stem cell transplants. • Effective options included oral hygiene, glutamine, vitamin E, aloe vera, olive oil, and laser therapy. • PBMT consistently eased pain and OM symptoms; glutamine reduced the incidence of OM in acute lymphoblastic leukemia patients (4.2% vs. 62.5%). • Oral cryotherapy and caphosol showed no benefit.
Braguês et al. 2024[27]	• OM is a frequent and painful side effect in children receiving antineoplastic therapy. • Many treatments have been proposed, but evidence on their effectiveness and safety in pediatric patients remains limited. • Interventions like PBMT, palifermin, honey, and zinc demonstrated preventive effects and symptom improvement. • Other promising approaches included cryotherapy and natural compounds. • Despite these options, current evidence remains insufficient to establish standardized clinical protocols.
Calarga et al. 2024[28]	• PBMT is safe and effective for treating and preventing OM in pediatric cancer patients, with no reported adverse effects. • Therapeutic PBMT reliably relieved pain, accelerated healing, and reduced OM severity and duration. • Preventive results were mixed, with some studies showing reduced incidence and others showing no benefit. • Protocol variability underscores the need for standardized guidelines and more high-quality research.
Campos et al. 2020[29]	• PBMT improved outcomes and reduced pain. • PBMT reduced the risk of severe OM by 64% (*RR*: 0.36; 95% *CI*: 0.29–0.44). • Cost-effectiveness analysis showed an ICER of 27.89 per severe OM case prevented, with an effectiveness gain of 132.2. • PBMT is both clinically beneficial and economically viable for OM management in head and neck cancer therapy.
Cronshaw et al. 2020[30]	• Larger optical spot sizes (0.51–4 cm^2) were linked to better clinical outcomes, especially for deeper tissue targets, compared to small spot sizes (0.02–0.08 cm^2). • Larger optical spot sizes are recommended for superficial and deep targets. • Higher surface doses are recommended to compensate for light scattering and ensure adequate target dosing. • Larger coverage saves time, allows higher total power at safe fluence, and delivers more energy to a greater tissue volume without toxicity.
Cronshaw et al. 2020[31]	• PBMT is a safe, effective option for OM in CT and head and neck RT. • There are no established universally optimized clinical protocols. • Best results were observed after pre-conditioning and concurrent use. • Wider beam coverage improves outcomes. • Pain management requires choosing between a focus on pain relief and a focus on healing.
Cruz et al. 2023[32]	• PBMT led to a more pronounced decrease in the severity of OM compared to control groups. • Significant differences across studies prevented a meta-analysis of outcomes related to lesion duration and pain relief.
de Oliveira et al. 2021[33]	• PDT + PBMT significantly reduced healing time compared to laser treatment alone ($p = 0.0005$). Photodynamic therapy showed considerable promise in treating OM, enhancing tissue regeneration and offering an effective approach for managing mucosal damage.
de Sales et al. 2025[34]	• PBMT reduced the incidence of OM in CT and RT patients, lowering IL-6 and TNF-α, increasing IL-4 and IL-10, and promoting tissue repair via antioxidant activity and keratinocyte maturation. • Benefits likely result from modulating inflammation, oxidative stress, and cellular repair; further research is needed to clarify mechanisms and optimize protocols.
Dipalma et al. 2024[35]	• PBMT effectively reduced the incidence of OM in patients undergoing CT/RT. • PBMT was associated with anti-inflammatory and antioxidant effects, including reduced IL-6 and TNF-α, and increased IL-4 and IL-10 levels. • The intervention promoted keratinocyte differentiation and tissue repair, suggesting its mechanism involves modulation of inflammation and oxidative stress.
Franco et al. 2023[36]	• The meta-analysis revealed a significant reduction in mucositis severity among patients treated with laser therapy compared to placebo (*SMD*: −1.34; 95% *CI*: −1.98–−0.98). • Laser therapy was effective in lowering the severity of OM caused by CT and radiation. • The findings support PBMT as a highly effective option for alleviating transplant-related mucositis symptoms.
Joseph et al. 2024[37]	• PDT + PBMT outperformed PBMT alone in healing and pain relief. • The meta-analysis (4 studies) showed a favorable effect of treatment (*SMD*: −0.51; 95% *CI*: −0.88–−0.15). • Combined therapy more effectively reduced OM severity in cancer patients. • PDT, especially when combined with PBMT, is a promising option for the management of OM symptoms.
Joseph et al. 2025[38]	• Out of 5 studies (256 participants), 4 reported that LED therapy reduced OM severity and pain; 1 study demonstrated faster healing. • LED therapy shows promise for OM symptom relief; larger, well-designed studies are needed to confirm its efficacy and optimize protocols.
Khalil et al. 2024[39]	• Four eligible studies used 660-nm InGaAlP diode lasers and showed low risk of bias. • PBMT consistently reduced the severity of OM across all studies. • Salivary cytokines assessed included IL-12p70, TNF-α, IL-6, IL-8, IL-10, CXCL8, and IL-1β, with IL-6 most strongly linked to OM severity. • Variability in cytokine levels was attributed to differences in cancer treatments and saliva collection methods.

Study	Main outcomes
Lai et al. 2021[40]	• Cryotherapy + PBMT was highly effective: none/mild OM (*OR*: 106.23); severe OM (*OR*: 0.01). • Cryotherapy alone: none/mild OM (*OR*: 3.13); severe OM (*OR*: 0.25). • PBMT alone: none/mild OM (*OR*: 7.56); severe OM (*OR*: 0.13). • Combined therapy outperformed cryotherapy or PBMT alone for none/mild OM (vs. PBMT *OR*: 14.06; vs. cryotherapy *OR*: 33.95). • No significant difference was noted for moderate OM. • All 3 approaches reduced severe OM; none showed advantage for moderate OM.
Parra-Rojas et al. 2025[41]	• Prophylactic PBMT effectively reduced the development and severity of OM in CT patients. • Red laser is more commonly used for intraoral PBMT, while infrared laser is preferred for extraoral applications. • Variability in PBMT protocols across studies makes it difficult to determine the ideal dosage. • PBMT is regarded as safe, non-invasive and free of side effects.
Peng et al. 2020[42]	• Preventive PBMT reduces the risk of OM in CT/RT patients. • Therapeutic PBMT may shorten the duration of severe OM. • Inconsistent outcomes across laser settings underscore the need to define optimal protocols.
Potrich et al. 2023[43]	• Cryotherapy + PBMT outperformed usual care, reducing severe OM and increasing none/mild cases. • The administration of cryotherapy or PBMT alone was effective but less so than combined therapy. • There were no differences among interventions for moderate OM. • Despite protocol variability, evidence supports both treatments, especially for severe OM.
Redman et al. 2022[44]	• PBMT may lessen OM severity and pain in children undergoing CT, though results are inconsistent. • The meta-analysis suggests benefit of the therapy, especially for severe OM, but significance was inconsistent. • Adverse events were rare and minor, though safety reporting was often poor. • Evidence is insufficient; more rigorous pediatric randomized controlled trials are needed.
Sánchez-Martos et al. 2023[45]	• PBMT reduced incidence and duration of severe OM (grades 3–4) in head and neck cancer patients during chemoradiotherapy. • Most studies reported significant pain reduction on VAS compared to controls. • PBMT improved oral health-related quality of life according to validated tools (e.g., EORTC QLQ-C30, UW-QoL). • PBMT is effective both for prevention and treatment, with benefits seen despite protocol variations.
Shen et al. 2024[46]	• PBMT significantly reduced the incidence of OM in cancer patients receiving CT/RT, as shown in pooled analysis across multiple trials. • Greater effectiveness was observed in pediatric patients compared to adults, with subgroup analysis supporting age-related differences in response. • No serious adverse effects related to PBMT were reported, indicating a strong safety profile. • Heterogeneity in treatment protocols and study designs underscores the need for standardized PBMT guidelines to optimize clinical outcomes.

CI – confidence interval; CT – chemotherapy; EORTC QLQ-C30 – European Organization for Research and Treatment of Cancer Quality of Life Questionnaire; ICER – Incremental Cost-Effectiveness Ratio; IL – interleukin; *OR* – odds ratio; *RR* – risk ratio; RT – radiotherapy; *SMD* – standardized mean difference; TNF-α – tumor necrosis factor alpha; UW-QoL – University of Washington Quality of Life Questionnaire; VAS – visual analogue scale.

Discussion

Background

Over the past 3 decades, photobiomodulation, now more precisely termed photobiomodulation therapy, has evolved from an intriguing laboratory observation to a modality incorporated into multiple international guidelines. The ensuing discourse synthesizes mechanistic insights, preclinical evidence, data from clinical trials, guideline positions, and implementation challenges, providing a panoramic view of the role of PBMT in the prevention and management of OM.[47–51] Radiotherapy, CT and high-dose conditioning regimens trigger a five-phase pathobiological cascade encompassing initiation, primary damage response, signal amplification, ulceration, and healing.[52] Reactive oxygen species and DNA strand breaks ignite necrosis factor kappa B (NF-κB)-mediated transcription of pro-inflammatory cytokines, notably tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6, which drive apoptotic loss of the basal epithelium and submucosal injury.[53,54] Secondary bacterial invasion further amplifies tissue damage, prolonging ulcerative phases and raising the risk of sepsis in neutropenic hosts.[55] Understanding these molecular checkpoints is critical, as PBMT targets several of the same signaling nodes, offering a biologically plausible strategy for interrupting OM evolution.[52–54]

Rationale for photobiomodulation therapy

Photobiomodulation therapy involves the delivery of photons (400–1100 nm) at low power (5–200 mW), which are primarily absorbed by mitochondrial cytochrome c oxidase.[55–62] From a photophysical perspective, blue photons possess higher quantum energy than red photons, a parameter that plays a fundamental role in determining the nature and efficiency of electromagnetic radiation interactions with biological matter. Blue (400–500 nm), red (620–750 nm) and near-infrared (750–1100 nm) light each exhibit distinct mechanisms of action in PBMT, reflecting differences in wavelength and tissue penetration depth. Blue light acts superficially (up to about 1 mm) and primarily exerts antibacterial and anti-inflammatory

effects by generating reactive oxygen species, which damage microbial cell membranes and modulate immune responses. Red light penetrates deeper (several millimeters) and activates mitochondrial cytochrome c oxidase, enhancing adenosine triphosphate (ATP) production, reducing oxidative stress, and stimulating tissue repair, angiogenesis and wound healing.[57–62] Near-infrared light reaches the greatest depths (up to several centimeters), affecting muscles and bones by activating cytochrome c oxidase, improving microcirculation, reducing inflammation, and promoting deep tissue regeneration. These differences guide clinical applications: blue light is used for superficial infections and inflammation; red light for soft tissue healing and pain reduction; and near-infrared light for musculoskeletal pain and deep tissue repair.[55–62]

The biological mechanisms and cellular effects triggered by PBMT include transient displacement of mitochondrial nitric oxide, which enhances oxidative phosphorylation and ATP synthesis.[63] Modulation of reactive oxygen species within a therapeutic window activates redox-sensitive transcription factors such as nuclear factor erythroid 2-related factor 2 (NRF2) without inducing oxidative stress.[64] The upregulation of growth factors like vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β) as well as increased collagen synthesis promote re-epithelialization.[65] The reduction of inflammatory cytokines including IL-6 and TNF-α in both saliva and serum, with levels correlating to mucositis severity scores, has also been observed.[39] Together, these actions produce analgesia, suppress inflammation, and accelerate wound healing, key outcomes in effective OM management. Studies on rodent models consistently show that PBMT administered at wavelengths of 660–810 nm and fluences of 2–6 J/cm^2 leads to smaller ulcers, faster epithelial regeneration, and reduced local TNF-α expression.[66–68] While animal studies employ standardized dosing conditions that do not fully reflect clinical variability, they serve to reinforce the mechanistic basis for PBMT and guide the selection of wavelength and fluence parameters in human trials.

Distinction between PBMT, PDT and combined applications

It is important to differentiate PBMT from PDT, as they differ in both mechanism of action and clinical application. Photobiomodulation involves the direct stimulation of tissue using low-level, non-ionizing radiation (typically in the red or near-infrared spectrum) and does not require a photosensitizing agent. The primary effects of PBMT include modulation of inflammation, enhancement of cellular repair, and acceleration of wound healing through mitochondrial and redox pathways.[69,70] In contrast, PDT requires the administration of a photosensitizer, which is subsequently activated by a specific wavelength of light. This activation leads to the generation of reactive oxygen species, resulting in selective cytotoxicity and tissue destruction, which is often used for antimicrobial or antitumor purposes.[69,70] While the mechanisms are distinct, several systematic reviews have noted that combining PDT with PBMT may provide added therapeutic benefits for patients with OM. For instance, de Oliveira et al. discovered that this combination resulted in significantly faster mucosal healing compared to the administration of PBMT alone.[33] Similarly, Joseph et al. reported that dual therapy produced greater reductions in pain and symptom duration than PBMT monotherapy.[37] These findings suggest that integrated protocols, leveraging the complementary actions of PDT and PBMT, may represent a promising direction for future management of mucositis. Nevertheless, further research is needed to optimize dosing and patient selection.[71]

Impact on quality of life

In addition to a reduction in the clinical severity and duration of OM, PBMT delivers consistent and measurable improvements to patient quality of life. This outcome has been documented in studies using validated instruments, including the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30), the University of Washington Quality of Life Questionnaire (UW-QoL), and the Functional Assessment of Cancer Therapy (FACT).[29,43,45] Photobiomodulation therapy supports key functional domains. After PBMT, patients report an improvement in their ability to eat, speak and perform daily activities. Preserved oral function contributes to improved nutritional status and a reduced risk of malnutrition. The alleviation in pain enables more restful sleep and facilitates social interaction and emotional stability. In controlled trials, PBMT-treated patients demonstrated significantly higher quality of life scores at the conclusion of treatment. For example, one study found UW-QoL scores of 687 for PBMT patients vs. 607 for placebo on day 35, with social-emotional scores also notably higher (408 vs. 348, $p = 0.003$).[45,72] These improvements translate to better psychological resilience, less disruption of cancer therapy, and reduced hospitalizations, underscoring the value of PBMT as a standard component of supportive oncology.

Impact on pain

Photobiomodulation has been shown to significantly reduce pain associated with OM in cancer patients, offering both preventive and therapeutic benefits. The included studies consistently report that PBMT reduces pain intensity, shortens lesion duration, and decreases the need for systemic analgesics, including opioids, particularly in patients undergoing head and neck chemoradiotherapy or HSCT.[28,29,36,45,46,65] The analgesic effects of the treatment are attributed to the modulation of inflammatory

cytokines such as IL-6 and TNF-α, stimulation of tissue repair, and improved microcirculation.[34,35,39] In clinical trials, PBMT-treated patients experienced up to a 50% reduction in mean pain scores compared with controls, enabling improved oral function, nutritional intake and quality of life.[45,65] Owing to its strong safety profile and non-invasiveness, the therapy is recommended as a standard supportive care intervention in international guidelines.[63,64]

Age-specific considerations and treatment protocols

The management of OM with laser therapy requires careful consideration of age-specific factors, as different patient populations present unique challenges and may respond differently to PBMT.[44,73] Evidence reveals significant variations in efficacy, tolerability and optimal protocols across age groups. In the case of very young children (3–6 years), the available data remains limited but promising, with cooperation being a key challenge.[73] For this age group, extraoral PBMT is often preferable to intraoral applications in order to minimize discomfort and reduce the need for active cooperation, with shorter session durations being recommended.[73] School-age children (7–12 years) have the strongest pediatric evidence base, with multiple systematic reviews confirming the efficacy of PBMT in this age group. A meta-analysis showed that prophylactic PBMT significantly reduced the odds ratio (*OR*) for developing OM (*OR* = 0.50; 95% confidence interval (*CI*): 0.29–0.87; $p = 0.01$) and severe mucositis (*OR* = 0.30; 95% *CI*: 0.10–0.90; $p = 0.03$).[74] School-age children generally cooperate well during intraoral applications under proper supervision, and standard protocols with 10–15-min sessions are well tolerated.[75] Adolescents and young adults (13–18 years) show excellent compliance and achieve outcomes similar to adults, with studies consistently reporting reduced severity and duration of mucositis. Photobiomodulation is considered an effective method for the treatment of OM in young cancer patients due to its analgesic, anti-inflammatory and healing properties.[76]

Adults represent the most extensively studied population for laser therapy in OM, with the strongest evidence base. The Multinational Association of Supportive Care in Cancer (MASCC) and the International Society of Oral Oncology (ISOO) have established guidelines recommending the use of 660-nm wavelength, a power density of 417 mW/cm^2, and an energy density of 4.2 J/cm^2 for patients receiving a combination of CT and RT.[63,77,78] In patients after HSCT, protocols using 660 nm with 2–4 J/cm^2 have been shown to result in a significant reduction in the incidence of severe mucositis. Elderly patients (>65 years) also benefit from laser therapy, with studies showing reductions in mucositis severity and duration, as well as a decrease in weight loss and lower morphine requirements. However, for this age group, slightly shorter sessions (10–20 min) and energy densities of 2–6 J/cm^2 with careful monitoring are advised.[79] Patients who had undergone HSCT, particularly adults, exhibit the most robust data, with meta-analyses showing significant reductions in the severity of mucositis. Pediatric HSCT patients demonstrate similar benefits, but modifications in session duration and technique are required.[80] For 5-FU-induced mucositis, limited age-specific data exists. The utilization of animal models has indicated that 660 nm and 6 J/cm^2 represent the optimal parameters.[81,82] Pediatric patients with methotrexate-induced mucositis respond particularly well to the treatment, with the incidence reduced from 66.67% to 6.67%.[73] Across all age groups, the optimal wavelengths range from 660 nm to 670 nm, with energy density and session length tailored to age, cooperation level and physiology: extraoral and brief sessions for very young children; supervised intraoral applications for school-age children; adult protocols for adolescents; standard intraoral treatment for adults; and modified positioning for elderly patients.[44] The safety profiles are favorable across all groups. A total of 2,700 patients were included in the analyzed studies, and minor, infrequent adverse events were observed. The majority of these events were associated with cooperation rather than device function. However, very young children may require sedation, while elderly patients may need the assessment for oral anatomy changes. Furthermore, all patients require eye protection during treatment.[44] Photobiomodulation protocols for edentulous individuals should ensure full coverage of the alveolar ridges and vestibular mucosa, as these areas are prone to mucositis-related ulceration and discomfort despite the absence of teeth. Moreover, proper adaptation of applicator angulation is essential to maintain consistent energy delivery.[80–82]

The implementation of laser therapy for OM patients should be guided by age-appropriate protocols, supported by targeted training that addresses developmental considerations and cooperation strategies. While current evidence supports the use of PBMT across all age groups, significant gaps remain, particularly with regard to very young children and elderly patients. Priority research areas include standardizing pediatric protocols across developmental stages, collecting long-term pediatric safety data, defining optimal approaches for elderly patients with comorbidities, clarifying age-dependent dose–response relationships, and developing strategies to enhance cooperation in young children. Acknowledging that a one-size-fits-all approach is not optimal, clinicians should tailor treatment to age-specific needs while promoting further research to refine these protocols.[44,75–82]

Xerostomia

The administration of daily PBMT with well-defined parameters reduces short-term OM symptoms in elderly

patients and improves salivary gland function. Oliveira et al. have demonstrated an increased salivary flow and protective potential of PBMT in the management of xerostomia.[83,84] Photobiomodulation acts by stimulating cytochrome c oxidase, activating cellular signaling pathways, increasing ATP production, enhancing metabolism, and providing anti-inflammatory and regenerative effects. Clinical trials have demonstrated that daily protocols of 810 nm at 6 J/cm^2 or 660 nm at 4 J/cm^2 result in a greater reduction in mucositis severity, pain and oral discomfort than every-other-day therapy. These findings are accompanied by an increased unstimulated and stimulated salivary flow, reduced xerostomia-related discomfort, and improved oral health-related quality of life by up to 52%, with effects that persist for up to one year.[83–87] These improvements in mucositis symptoms may be enhanced by positive effects on salivary gland function.[87] The standard protocol outlined by Ferrandez-Pujante et al. involves 6 weekly sessions over a period of 6 weeks, with extraoral application over the salivary glands at 810 nm and 6 J/cm^2 using a GaAlAs diode, with a duration of 2 min 24 s for the parotid gland and 1 min 12 s for the submandibular gland.[88] Lončar et al. describe an intensive protocol of 10 consecutive daily sessions applying PBMT parameters of 904 nm at 246 mW/cm^2 and 29.5 J/cm^2 for 120 s per area, both extraorally and intraorally at sublingual glands.[89,90] Clinical studies in older adults have reported an increased unstimulated and stimulated salivary flow, reduced subjective oral dryness, and a 52% improvement in oral health-related quality of life.[89,90] These therapeutic effects were maintained for up to 1 year.[88,89] The benefits have been linked to the regeneration of salivary gland cells, improved microcirculation, increased salivary immunoglobulin A, and reduced oxidative stress.[87,90,91] A meta-analysis conducted by Oliveira et al. confirmed these effects, identifying optimal parameters as wavelengths of 790–830 nm, power of 30–120 mW, energy density below 30 J/cm^2, and 2 to 3 weekly sessions.[83] Significant increases were noted in stimulated salivary flow (mean difference (*MD*) = 2.90; 95% *CI*: 1.96–3.84), and reductions were observed in xerostomia-related pain (*MD* = −3.02; 95% *CI*: −5.56–−0.48). Furthermore, an enhancement in the quality of life was documented.[83,92] Benefits extend to elderly patients suffering from post-RT hyposalivation.[88–92]

Clinical applications

Preventive PBMT, when initiated on the first day of chemoradiation or conditioning, reduces the incidence of grade 3–4 OM by approx. 40–80% compared with sham treatment or standard oral care.[65,93,94] Therapeutic PBMT, applied at the onset of OM, shortens ulcer duration by 4–7 days and reduces mean pain scores by half on validated assessment scales.[95,96] Patients receiving PBMT also require substantially less systemic opioid use, highlighting its analgesic benefit.[65,97] In the HSCT setting, low-power 660-nm diode lasers (4 J/cm^2 intraorally) have reduced the incidence of severe OM from 66.67% to 6.67% in pediatric patients, demonstrating notable efficacy even in profoundly myelosuppressed hosts.[96] A meta-analysis of 3 randomized controlled trials in adults undergoing myeloablative transplants reported a standardized *MD* of −1.34 (95% *CI*: −1.98–−0.98) for severe OM, favoring PBMT.[36,95] In head and neck RT, a landmark French multicenter trial demonstrated that a daily 632.8-nm helium–neon (He–Ne) laser treatment (2 J/cm^2 prophylactic; 4 J/cm^2 therapeutic) during concurrent chemoradiotherapy reduced grade 3–4 OM incidence to 6.4% compared with 48% in the placebo group, and decreased gastrostomy placement rates and unplanned treatment interruptions.[65] Follow-up studies using 650-nm LED arrays and 850-nm extraoral panels have replicated these outcomes while improving clinical workflow.[95–100] Among patients receiving solid tumor CT, particularly 5-FU-based regimens, results varied but generally demonstrated a relative risk reduction of 23–28% in severe OM during weeks 3–4 of treatment, likely due to shorter duration of PBMT and non-standardized energy delivery.[93,100,101]

Possile confounding factors

When interpreting the findings of this umbrella review, it is important to recognize the potential influence of confounding factors that may have affected the reported efficacy of PBMT for OM. Variations in cancer type, disease stage and oncologic regimens (including CT agents, RT dose and field, and conditioning protocols for HSCT) can substantially alter OM risk and severity, thereby influencing the apparent benefit of PBMT. Patient-related factors, such as age, sex, comorbidities, nutritional status, and baseline oral health, also represent important sources of variability.[25–46] Furthermore, concomitant supportive care interventions, including mouth rinses, cryotherapy or pharmacologic agents, may act synergistically or independently to reduce OM severity, making it difficult to isolate the effect of PBMT. The technical heterogeneity in laser parameters, such as wavelength, fluence, power density, application technique, frequency, and intraoral vs. extraoral delivery, further complicates the comparison of results across studies. Differences in operator experience, treatment setting and adherence to standardized protocols add an additional layer of complexity. The presence of these confounders, whether individually or in combination, has the potential to introduce bias into effect estimates. Consequently, it is essential to consider these confounders when interpreting the results of pooled studies and formulating clinical recommendations.[25–46]

International guidelines and consensus positions

International bodies have progressively upgraded PBMT recommendations (Table 5). The 2020 MASCC/ISOO guidelines categorize intraoral PBMT as level I (strong)

Table 5. Summary of international guidelines for photobiomodulation therapy (PBMT)

Guidelines	Year	Target cohorts	Recommendation	Reference
MASCC/ISOO	2020	HSCT, head and neck cancer, RT, CT	intraoral PBMT for prevention	63
NICE IPG615	2018	RT/CT mucositis	PBMT acceptable with audit	102
WALT	2022	HSCT, RT, CT	use of prescriptive PBMT parameters	64
HTW EAR044	2022	Welsh NHS patients	support PBMT, emphasis on clinician training	103

HSCT – hematopoietic stem cell transplantation; HTW – Health Technology Wales; ISOO – International Society of Oral Oncology; MASCC – Multinational Association of Supportive Care in Cancer; NHS – National Health Service; WALT – World Association for Photobiomodulation Therapy.

for the prevention of OM in HSCT and head and neck RT, contingent on adherence to validated protocols.[63] The National Institute for Health and Care Excellence (NICE) Interventional Procedures Guidance 615 (IPG615) found "adequate efficacy and no major safety concerns," supporting the adoption of PBMT in UK centers with governance oversight.[102] The World Association for Photobiomodulation Therapy (WALT) 2022 position paper extends PBMT to additional indications such as radiodermatitis and lymphedema, stipulating explicit dosimetry ranges.[64] The 2022 Health Technology Wales (HTW) appraisal (EAR044) supported PBMT implementation across the Welsh National Health Service (NHS), emphasizing the importance of clinician training and service evaluation.[103]

According to Parker et al., the complete and precise documentation of all laser operating parameters is a fundamental prerequisite for the reproducibility and scientific validity of laser–tissue interaction studies.[104] The authors emphasize that the reporting of only basic information such as output power or wavelength is insufficient. Instead, a comprehensive set of parameters should be included, such as the total energy delivered [J], energy density (fluence) [J/cm^2], power density (irradiance) [W/cm^2], irradiation time [s], pulse repetition rate [Hz], beam diameter at the target [cm], mode of application (contact or non-contact), beam divergence angle [°], emission mode (continuous wave, pulsed), number and frequency of treatment sessions, and distance from the tip to the tissue. The omission of these variables not only compromises clinical reproducibility but also increases the risk of thermal side effects or treatment failure.[80] While no universal gold standard exists, multiple positive randomized controlled trials converge on the use of red (630–680 nm) or near-infrared (780–850 nm) light, with prophylactic fluence around 2 J/cm^2 and therapeutic doses of 4–6 J/cm^2, typically applied daily or on alternate days.[64,65] Extraoral panels offer ergonomic advantages but require a longer exposure time of approx. 15 min to overcome cutaneous attenuation.[99] Effective PBMT covers the lips, buccal mucosae, ventral tongue, floor of mouth, and soft palate, administered in a grid of 1-cm^2 points. A review of studies involving over 1,000 patients has revealed no association between PBMT and carcinogenicity, tumor promotion, or serious adverse events, with only mild, transient sensations such as warmth or a metallic taste occasionally reported.[65] However, it should be noted that there are several limitations in the current evidence base. Parameter heterogeneity, such as inconsistent wavelengths, energy densities and treatment grids, compromises the comparability of meta-analyses. Blinding presents a challenge, because patients may perceive active treatments as different to sham treatments, potentially resulting in biased subjective pain outcomes. While robust data exists for adult HSCT populations, pediatric dosing, particularly in infants and toddlers, is still largely empirical.[19,105–108] Future research should focus on precision dosimetry using real-time optical feedback and Monte Carlo-guided fluence planning to tailor therapy to individual mucosal thickness and pigmentation. Furthermore, studies should explore synergistic PBMT combinations with agents like benzydamine or probiotic lozenges for broader mucosal protection, investigate biomarker-guided timing based on salivary cytokine thresholds such as IL-6, and develop automated extraoral LED devices, like wearable neck collars, for home-based PBMT prophylaxis during chemoradiation.[101] Photobiomodulation has transitioned from experimental therapy to evidence-based standard of care for high-risk OM populations. Its multimodal benefits, including analgesia, anti-inflammation and epithelial repair, address the full spectrum of OM pathobiology with minimal toxicity. In order to realize the full clinical and economic potential of PBMT, there is a necessity for adherence to validated dosimetry parameters and integration into multidisciplinary supportive care pathways. Further harmonization of protocols, expansion into pediatric and immunotherapy cohorts, and monitoring of long-term oncologic outcomes will serve to consolidate the essential role of PBMT in the management of mucositis.[104–108]

The international consensus documents provide precise parameters for PBMT, namely wavelength, power density, fluence, timing, and field mapping, for the purposes of OM prevention and treatment (Tables 6–8). The application of these validated settings is critical, and deviating from them markedly reduces treatment efficacy.

MASCC/ISOO 2020 guidelines – dose

In the MASCC/ISOO 2020 guidelines,[63] evidence-based protocols are linked to the specific oncologic treatment settings (Table 6). Each row represents a complete, validated regiment, and the listed parameters should not be interchanged across protocols.

Table 6. Multinational Association of Supportive Care in Cancer (MASCC)/International Society of Oral Oncology (ISOO) 2020 guidelines for the use of photobiomodulation therapy (PBMT)[63]

Cancer setting	λ [nm]	Power density [mW/cm^2]	Time point [s]	Fluence [J/cm^2]	Spot size [cm^2]	Sites treated, *n*	Application time
HSCT (adult)	632.8	31.25	40	1.0	0.80	18	D +1 to D +5 post-conditioning
HSCT (pediatric & adult)	650.0	1000.00	2	2.0	0.04	54–70	D +1 to D +2 (7–13 D total)
H&N RT (alone)	632.8	24.00	125	3.0	1.00	12	entire RT course
H&N RT–CT	660.0	417.00	10	4.2	0.24	72	entire RT–CT course
H&N RT–CT	660.0	625.00	10	6.2	0.04	69	entire RT–CT course

H&N – head and neck cancer; D – day.

Table 7. World Association for Photobiomodulation Therapy (WALT) position paper 2022 guidelines for the use of photobiomodulation therapy (PBMT)[64]

Indication and delivery	Wavelength band [nm]	Power density [mW /cm^2]	Photon fluence per field [E]	Session frequency	Notes
Prevention – intraoral	630–680	10–50	1.2 (≈ 5.7 pJ /cm^2)	pre-oncotherapy once daily ≤120 min	non-thermal application (<45°C)
Treatment – intraoral	630–680	10–50	2.5 (≈ 11.4 pJ /cm^2)	3–4 times per week until healing	typically 15–20 sessions
Prevention – transcutaneous	800–1,100	30–150	1.0 (≈ 4.5 pJ /cm^2)	pre-oncotherapy once daily for 30–120 min	face/neck panel application
Treatment – transcutaneous	800–1,100	30–150	1.3 (≈ 9 pJ /cm^2)	3–4 times per week until healing	typically 15–20 sessions

WALT position paper 2022 – consensus operating windows

In the 2022 WALT position paper,[64] the available evidence is synthesized into 2 pragmatic device classes. Photon fluence is expressed in Einsteins [E] (1 E ≈ 4.8 pJ/m^2 at 810 nm) (Table 7).

NICE IPG615 2018 – service-level guidance

Although NICE does not mandate particular dosimetry settings, IPG615 outlines the procedural standards that must guide the clinical use of PBMT within the UK healthcare system (Table 8).[102]

Long-term safety of PBMT

Long-term safety data for PBMT in treating OM in cancer patients demonstrate that PBMT is safe when applied in accordance with the current clinical guidelines. There is no evidence to suggest that PBMT increases the risk of secondary malignancies or tumor recurrence in the oral cavity.[109–112] A 15-year retrospective study in HSCT patients found no immediate or late adverse effects, including no secondary oral cancers linked to PBMT protocols for OM management.[109] Similarly, a systematic review of PBMT use for cancer therapy-related toxicities, including OM, reported no tumor safety concerns or significant side effects attributable to PBMT.[110] Prospective and retrospective clinical trials in head and neck cancer and HSCT consistently show excellent safety and tolerability, with no device-related adverse events observed across hundreds of treatment sessions.[111,112] These studies confirm the absence of both acute and chronic adverse effects, reinforcing a favorable long-term safety profile of PBMT. According to the WALT guidelines, PBMT should not be applied directly over active tumor sites, despite the absence of evidence indicating tumor promotion.[64] While PBMT effectively reduces the severity and pain associated with OM, further research is needed to standardize dosimetry and treatment protocols. The current body of evidence supports the continued use of PBMT for OM in cancer patients without identified long-term safety concerns.[104,109–120]

Future directions

Future perspectives in PBMT highlight the need for further research to refine and expand its clinical applications.

Table 8. National Institute for Health and Care Excellence (NICE) IPG615 2018 guidelines for the use of photobiomodulation therapy (PBMT)[102]

Parameter	Recommendation
Light range	red or near-infrared spectrum
Delivery route	intraoral or extraoral probe, or a combined approach
Session duration	20–30 min per treatment
Frequency	2–5 sessions per week throughout oncologic treatment
Starting point	prior to CT/RT to prevent OM
Governance	use within local clinical audit and training pathways; no major safety concerns identified

Emerging studies emphasize clarifying the precise cellular and molecular mechanisms by which PBMT modulates biological processes, including mitochondrial function, redox signaling and gene expression. The integration of real-time monitoring with artificial intelligence has the potential to optimize dosing in accordance with the individual characteristics of each patient's tissue. Additionally, advances in technology that combine PBMT with nanomaterials and biomaterials hold the potential to enhance targeting and therapeutic outcomes. The standardization of clinical protocols through rigorous trials remains essential to ensure reproducibility, efficacy and safety across diverse patient groups.

Conclusions

This umbrella review consolidates robust evidence demonstrating that PBMT is a clinically effective and safe approach for the prevention and treatment of OM across various oncologic settings. Supported by findings from 22 high-quality systematic reviews and according to the international guidelines (MASCC/ISOO, WALT, NICE), PBMT significantly reduces the incidence, severity, pain, and duration of OM, while concomitantly improving patients' quality of life. Furthermore, it facilitates uninterrupted cancer therapy by minimizing treatment-related complications. The Polish Society for Laser Dentistry strongly endorses PBMT as a standard component of supportive care for high-risk patients, especially those undergoing HSCT or head and neck chemoradiotherapy. Preventive PBMT protocols should be initiated before or at the beginning of therapy using validated dosimetry parameters. In order to ensure both the efficacy and reproducibility of treatment, it is imperative to adhere to the established technical standards. Future efforts should focus on harmonizing pediatric protocols, improving access to PBMT in clinical practice, and advancing research into optimized delivery, biomarker-guided timing and combination therapies. Photobiomodulation constitutes a transformative, evidence-based solution that addresses a critical gap in oncology supportive care, with clear benefits for patients and healthcare systems alike.

Consensus-based clinical guidance from the Polish Society for Laser Dentistry

Based on the synthesis of 22 high-quality systematic reviews and meta-analyses, as well as in alignment with the international guidelines (MASCC/ISOO, WALT, NICE), the PTSL endorses the use of PBMT as a safe and effective modality for the prevention and treatment of OM in patients with head and neck cancer. This recommendation applies to both adult and pediatric populations. However, in very young children or patients who cannot tolerate intraoral application, extraoral (near-infrared) protocols are preferred.

Key clinical recommendations:

- PBMT should be incorporated as standard supportive care for patients receiving high-risk cancer therapies, including HSCT and head and neck chemoradiation;
- Preventive PBMT, initiated before or on the first day of oncologic therapy, significantly reduces the incidence and severity of OM and should be prioritized;
- Implementation should be coordinated with the treating oncologist, who serves as the main clinician overseeing the patient's oncologic management;
- Therapeutic PBMT, applied after the onset of mucositis, offers clinically meaningful analgesia and accelerates healing;
- Recommended parameters include: wavelengths of 630–670 nm (intraoral) and 780–850 nm (extraoral); fluence of 2–6 J/cm^2; and frequency of 3–4 times/week or daily throughout the treatment course (Tables 9,10);
- Treatment should cover both intraoral and extraoral sites, including the lips, buccal mucosa, ventral tongue, floor of the mouth, and soft palate. In acute cases, the protocol should be extended to regional lymph nodes to address lymphatic congestion and reduce inflammation.

Photobiomodulation protocols must strictly follow validated dosimetry guidelines to ensure reproducibility and clinical benefits. Deviations from evidence-based parameters markedly reduce treatment efficacy. While the safety profile is excellent, standardized training and documentation are essential for clinical integration. Further efforts should be made to harmonize the use of PBMT in pediatric settings and to explore its cost-effectiveness across various healthcare systems.

Table 9. Typical photobiomodulation therapy (PBMT) parameters by patient population for the prevention and treatment of oral mucositis (OM)

Population	Dosing regimen	Wavelength [nm]	Power and energy density	Exposure time [min]	Frequency	Application method
Pediatric (3–6 years)	short sessions; extraoral PBMT preferred	630–670 (intraoral) 780–850 (extraoral)	5–200 mW/cm^2 2 J/cm^2 (prophylactic) 4–6 J/cm^2 (therapeutic)	5–10	daily or 3–5 times per week	extraoral for young children; intraoral for older children
Adults	standard adult protocols	660–670 (red)	~417 mW/cm^2 2 J/cm^2 (prophylactic) 4.2 J/cm^2 (therapeutic)	10–15	daily or on alternate days	intraoral
Geriatric (>65 years)	slightly shorter sessions (10–20 min)	630–670 (intraoral) 780–850 (extraoral)	2–6 J/cm^2 (energy density)	10–20	daily or 3–5 times per week	intraoral and extraoral depending on access and patient comfort; modified positioning to ensure mucosal coverage

Table 10. Recommended operating ranges for photobiomodulation therapy (PBMT) in the prophylactic and therapeutic management of oral mucositis (OM), based on the MASCC/ISOO 2020, WALT 2022 and NICE 2018 guidelines[63,64,102]

Parameter	Recommended range for prophylactic use	Recommended range for therapeutic use	Notes
Wavelength [nm]	630–670 (intraoral) 780–850 (extraoral)	630–670 (intraoral) 780–850 (extraoral)	choice depends on the required depth of tissue penetration
Power density (irradiance) [mW/cm^2]	5–100	5–200	lower ranges are suitable for superficial tissues; higher ranges for deeper targets
Energy density (fluence) [J/cm^2]	~2	4–6	surface doses may need adjustment based on tissue depth and optical attenuation
Exposure time [min]	5–10	10–20	longer exposure is often required for extraoral application
Frequency	daily or 3–5 times per week	daily or on alternate days	treatment duration and frequency should be tailored to the clinical indication
Emission mode	continuous wave or pulsed	continuous wave or pulsed	mode selection depends on device capabilities and treatment goals

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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Review

Sex difference in the hyoid bone position in adults with obstructive sleep apnea: Systematic review and meta-analysis

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Abstract

The hyoid bone exhibits potential sex-based variations and is implicated in the severity of obstructive sleep apnea (OSA). Sex-specific comparisons are lacking. The present meta-analysis aimed to address this gap.

The Embase, MEDLINE and Web of Science databases were searched. The inclusion criteria were as follows: studies that reported the measurements of the hyoid bone–mandibular plane distance (HMP), demonstrated in cephalometric imaging (CEPH) in patients with OSA of both sexes, involving a polysomnography (PSG) examination with the apnea–hypopnea index (AHI), as well as information on the body mass index (BMI) and age. The exclusion criteria comprised reviews, meta-analyses and case reports. The risk of bias was assessed with the use of the Scottish Intercollegiate Guidelines Network (SIGN) checklist. Statistical analysis was conducted using Comprehensive Meta-Analysis software (CMA) and IBM SPSS Statistics for Windows.

Seven observational studies with 718 adult patients (515 males and 203 females) met the inclusion criteria. The mean HMP value was 20.5 ±3.8 mm, with a significant difference observed between males (21.6 ±3.3 mm) and females (17.8 ±3.7 mm) ($p < 0.00001$). The correlation between HMP and AHI was significantly stronger in females – 2.5 times higher than in males ($r = 0.423$ vs. $r = 0.167$, respectively).

Although a standard range of the hyoid bone position for healthy adults and elderly individuals is currently lacking, sex significantly affects the anatomical variation of the hyoid mandibular position in patients with OSA. It is crucial to identify distinct OSA endotypes by sex to ensure accurate diagnosis and treatment planning, which could lead to sex-specific therapeutic strategies.

Keywords: obstructive sleep apnea, sex differences, hyoid bone position, cephalometric imaging

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Highlights

- The research indicates that the hyoid bone–mandibular plane distance (HMP) is significantly influenced by sex in individuals diagnosed with obstructive sleep apnea (OSA).
- On average, males exhibited a greater HMP value (21.6 ±3.3 mm) as compared to females (17.8 ±3.7 mm).
- These findings suggest that sex-related anatomical variations play a crucial role in the positioning of the hyoid bone, which may have diagnostic and therapeutic implications in the management of OSA.

Introduction

Sex-based medicine is a branch of medical science that focuses on understanding and addressing the physiological and biological distinctions between males and females.[1,2] This field recognizes that the biological differences associated with sex, including anatomical variations and genetic factors, can significantly impact health outcomes and responses to medical treatment.[2,3] Sex-based medicine advances our understanding of how these differences influence disease manifestation and progression, and treatment efficacy.[1] By considering sex-specific factors in research, diagnosis and treatment protocols, healthcare professionals aim to provide more tailored and effective medical care for both men and women.[2,4]

Some evidence suggests significant sex-based differences in the hyoid bone position,[5] morphology[6] and volume.[7] The hyoid bone is a unique, horseshoe-shaped structure situated in the anterior midline of the neck, constituting a distinctive feature in the human skeletal system.[8] Unlike other bones, the hyoid bone does not articulate with any other bone. Instead, it is suspended by ligaments and muscles.[8,9] It is comprised of a central body and 2 pairs of processes extending from its ends – the greater and lesser horns. The hyoid bone is a crucial anchor for various muscles and ligaments involved in the intricate biomechanics of the head and neck.[8,9] It also plays a vital role in supporting the upper airway, as it is positioned at the 3rd cervical vertebra (C3) level. It contributes significantly to essential functions, such as speech, mastication and swallowing.[10]

Obstructive sleep apnea (OSA) is the most common sleep disorder in the adult population (prevalence of 6–17%).[11] Men have a higher rate of OSA than women. This sex difference persists even when accounting for the age and body mass index (BMI) differences between men and women. The risk of OSA increases with age for both sexes.[12]

Obstructive sleep apnea involves a decrease or complete halt in airflow despite an ongoing effort to breathe.[12] It occurs when the muscles at the floor of the mouth, the suprahyoid muscles (including mylohyoid, geniohyoid, digastric, and stylohyoid muscles), relax during sleep, causing the soft tissue in the throat to collapse and block the upper airway.[12] The etiological factors of OSA include the craniofacial anatomical features, such as mandibular size, mandibular body length and the tongue volume,[13] the accumulation of fatty tissue in the parapharyngeal area and increased body weight, which may decrease the upper airway diameter, thus favoring its collapse.[13] Impaired neural control and upper airway myopathy can also increase the risk of OSA.[14] Untreated OSA is related to 5 major cardiovascular diseases: hypertension; heart failure; atrial fibrillation; coronary artery disease; and stroke.[15–17] Recently, it has been found that different biomolecules (calcium (Ca), magnesium (Mg), vitamin D, and uric acid), as well as serum neuronal PAS domain protein 2 (NPAS2) metabolic dysregulation may be implicated in the OSA etiology.[18,19]

The gold standard for diagnosing OSA is a sleep study.[12] The apnea–hypopnea index (AHI) classifies the severity of OSA into 3 categories: mild; moderate; or severe.[20,21] Several imaging methods, such as video fluoroscopy, cephalometric imaging (CEPH), computed tomography (CT), and magnetic resonance imaging (MRI), static or dynamic, may aid clinicians in better recognizing the individual craniofacial anatomical features that may be the cause of OSA, thus helping in diagnosis and personalized treatment planning.[22] Each kind of imaging presents advantages and disadvantages. Cephalometric imaging remains a valuable tool for assessing skeletal structures and providing an initial evaluation of the upper airway.

One of the anatomical features, the inferior-dorsal position of the hyoid bone, which is measured via CEPH,[23–25] and expressed as the hyoid bone–mandibular plane distance (HMP) in millimeters, is suggested to be of importance in some patients with OSA.[26–28] One of the parameters for measuring the hyoid bone position in CEPH is the perpendicular distance from the most superior-anterior point on the body of the hyoid bone (H) to the mandibular plane (MP), which is constructed by connecting the lowest point on the lower border of the mandibular body (gnathion) and the lowest point on the lower border of the mandibular ramus (gonion), the reference point on the C3 being the most inferior-anterior point on the body of the C3 (Fig. 1).[29] The average HMP value in the healthy population is 9.03 ±3.92 mm, whereas it amounts to 22.81 ±6.76 mm in OSA patients.[30] It has been found that HMP is longer in patients who suffer from severe OSA as compared to those who do not.[31]According to a meta-analysis conducted by Neelapu et al., there is an average difference of 4.0–6.6 mm in HMP between patients with

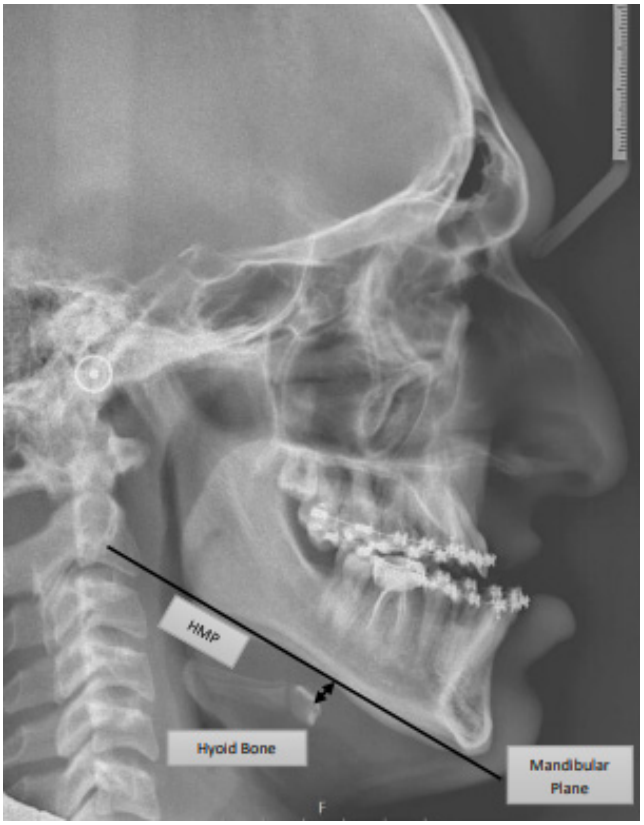

Fig. 1. Hyoid bone–mandibular plane distance (HMP)

OSA and those without it (controls), with OSA patients having a longer HMP.[28]

Surprisingly, the abovementioned meta-analysis and other cited studies did not perform any comparison between males and females,[28,30,31] even though several significant sex-based differences in the OSA prevalence, clinical presentation and management are supported by research.[32]

Considering the fact that sex plays a role in OSA and affects the hyoid bone position, as well as the reported greater HMP values among patients with OSA, we investigated in the present systematic review and meta-analysis whether there were significant sex-related differences in the hyoid bone position in patients with OSA.

Methods

We developed a review protocol according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement.[33] The study protocol was registered with PROSPERO before initiating this systematic review and meta-analysis (ID: CRD42023446388).

Identification and selection of studies

The electronic databases Embase, MEDLINE and Web of Science were searched with regard to the period from 1946 to February 2023.

A comprehensive search was conducted across the databases on February 15, 2023, focusing on the 'hyoid bone' and its association with sleep-disordered breathing, including 'sleep apnea.' The queries yielded 637 results from Embase, 473 results from MEDLINE and 358 results from the Web of Science. Thus, there was a total of 1,468 initial records. After removing duplicates, 832 unique records were identified and uploaded into the Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia; https://www.covidence.org). Two independent reviewers (D.G.-A. and A.E.-P.) screened the titles and abstracts of all the articles to assess the eligibility of each study.

Eligibility criteria

To be eligible, a study had to be in English; it had to be an observational, cross-sectional or clinical study reporting HMP in OSA patients of both sexes, using CEPH; it had to involve a polysomnography (PSG) examination (at a cut-off value of the apnea–hypopnea index (AHI) ≥5), and provide information on the patients' BMI, age and sex. Reviews, meta-analyses and case reports were excluded. Both reviewers performed the screening and assessments, discussing their progress and decisions.

Outcome measures

The outcome measures in this study were AHI as a means to define the presence of OSA and CEPH as a means to determine HMP in millimeters.

Data extraction

After extracting the data, we further disqualified articles by reading the titles and abstracts. In the next step, the reviewers read the texts in full. In case of disagreement, they discussed the issues with a third reviewer (T.G.). Authors' names, the journal name, the year of publication, the country, and the method used to diagnose OSA were registered. The reviewers analyzed the following parameters: the sample size (the total number of cases with OSA); the patients' mean age and their sex (the number of male and female patients suffering from OSA); the patients' mean BMI; and the mean HMP for each sex. To evaluate the correlation between HMP and OSA for both sexes, statistical analysis was performed, as detailed below.

Assessing the risk of bias

To assess the risk of bias, we used the NIH quality assessment tool – the Scottish Intercollegiate Guidelines Network (SIGN) checklist (National Institutes of Health (NIH), Bethesda, USA) to estimate the quality of observational cohort and cross-sectional studies (supplementary material available from the corresponding author

on request). Two independent reviewers (D.G.-A. and A.E.-P.) employed the following methodological criteria to assess the risk of bias in each of the eligible studies: the research question or objective clearly stated; the population specified and defined; a participation rate of eligibility (>50%); clear inclusion/exclusion criteria; sample size justification; the exposure(s) of interest measured prior to the outcome(s) being measured; the timeframe sufficient for an association between the exposure and the outcome; the examination of different levels of the exposure as related to the outcome; the definition of the exposure measures, and their validity and reliability; the binding of the exposure to the definition of the outcome measures, and their validity and reliability; and the measurement and statistical adjustment of the critical potential confounding variables.

Each reviewer completed the SIGN/NIH checklist for the included studies, and determined the overall risk of bias, rating it as low (a score of 9–12 methodological points), moderate (a score of 5–8 methodological points) or high (a score of 0–4 methodological points). Any disagreement was resolved through discussion with a third reviewer (T.G.). The reviewers contacted the authors of the publications for clarification in case of unclear or missing information.

Statistical analysis

Meta-analysis was performed using primary outcome measures when there were 5 or more studies with a low to moderate risk of bias, and similar assessment and measurement techniques. The results for the eligible studies were pooled using the Cochrane ReviewManager (RevMan; https://training.cochrane.org/online-learning/core-software) via a random effect. The one-way analysis of variance (ANOVA) was conducted using the Comprehensive Meta-Analysis software (CMA), v. 4.0 (Biostat, Inc., Englewood, USA; https://meta-analysis.com). This analysis examined differences in the position of the patients' hyoid bone by sex, considering additional parameters, such as AHI, BMI and age. In addition, an attempt was made to examine differences in those parameters between the patients with OSA and those without OSA; however, due to the paucity of studies regarding the population without OSA, it was impossible to conduct a statistical analysis with sufficient validity and power.

We built a data model for multivariate analysis using IBM SPSS Statistics for Windows, v. 23.0. (IBM Corp., Armonk, USA), applying the weight function according to the number of observations in each study and each sex group. Some of the one-way tests were repeated with a tool more familiar to the researcher (IBM SPSS Statistics for Windows) to analyze the findings previously examined in CMA. Spearman's correlations were determined with regard to HMP, AHI and BMI for each sex separately. Then, a generalized linear model analysis was performed for HMP as a dependent variable based on AHI, BMI, age, and sex.

Confidence in cumulative evidence

For each outcome, we evaluated the level of confidence in the evidence accumulated from all sources by following the guidelines outlined in GRADE.[34–38] We conducted a comprehensive assessment, considering various factors, such as the risk of bias, the consistency of the results, the effect size, and the sample size. Based on this analysis, we assigned each outcome an overall confidence level – high, moderate, low, or very low.

Results

Study selection

The literature search yielded 1,468 studies, of which 636 duplicates were removed, leaving 832 studies that were screened by title and abstract. After that, only 158 studies remained relevant. After reading the texts in full, 7 articles were selected and included in the present study. Out of the 151 studies that were rejected based on full text, the majority were excluded due to wrong patient population, outcome and intervention. The review process flow chart is shown in Fig. 2.

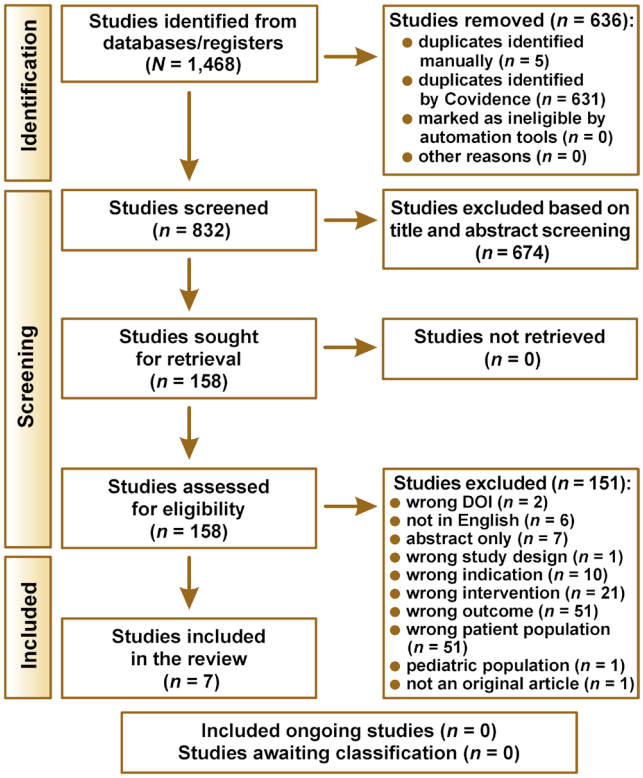

Fig. 2. Flow chart of the study according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement

Study characteristics

The eligible studies included in the analysis were all observational and cross-sectional, except for one retrospective cohort study.[39] They used lateral cephalometric radiographs as an outcome measure for patients with OSA. The characteristics of each study are presented in Table 1.

Assessment of the risk of bias

This systematic review assessed the risk of bias in each study. Among the cross-sectional and cohort studies, 6 studies were rated as having a low risk of bias,[40–45] while one was rated as having a moderate risk of bias (Table 2).[39] However, none of the studies provided details

Table 1. Characteristics of the included studies

Study	Design	Participants (patients with OSA)	Measurement instrument	Outcome measure
de Tarso Moura Borges et al.[39] 2015	cohort study	102 57 M/45 F	PSG CEPH	the severity of OSA measured based on AHI (AHI ≥ 5) HMP by PSG
Amitani et al.[40] 2020	cohort study	112 56 M/56 F	PSG CEPH	HMP by PSG
Tuna et al.[41] 2012	cohort study	93 71 M/22 F	PSG CEPH	AHI HMP by PSG
Chang and Shiao[42] 2008	cross-sectional study	99 84 M/15 F	PSG CEPH	AHI HMP by PSG
An et al.[43] 2020	cohort study	89 55 M/34 F	PSG CEPH	AHI HMP by PSG
Hsu et al.[44] 2005	cohort study	65 57 M/8 F	PSG CEPH	OSA defined in a dichotomous way (the cut-off value of AHI = 5 discriminated between OSA and non-OSA) HMP by PSG
Cho et al.[45] 2019	cross-sectional study	158 135 M/23 F	PSG CEPH	the cut-off point for OSA was AHI ≥ 5 HMP by PSG

OSA – obstructive sleep apnea; M – male; F – female; PSG – polysomnography; CEPH – cephalometric imaging; AHI – apnea–hypopnea index; HMP – hyoid bone–mandibular plane distance.

Table 2. Risk of bias assessment

Study	Research question or objective clearly stated	Population specified and defined	Participation rate of eligibility (>50%)	Clear inclusion and exclusion criteria	Sample size justification	Exposure(s) of interest measured prior to the outcome(s) being measured	Timeframe sufficient for an association between the exposure and the outcome	Examination of different levels of the exposure as related to the outcome	Definition, validity and reliability of the exposure measures	Definition, validity and reliability of the outcome measures bound to the exposure	Outcome assessors blinded to the exposure	Key potential confounding variables measured and statistically adjusted	Total risk of bias score out of 12
de Tarso Moura Borges et al.[39] 2015	yes	yes	yes	yes	no	yes	no	yes	yes	no	no	yes	8
Amitani et al.[40] 2020	yes	yes	yes	yes	yes	yes	no	no	yes	yes	no	yes	9
Tuna et al.[41] 2012	yes	yes	yes	yes	no	yes	no	yes	yes	yes	yes	yes	10
Chang and Shiao[42] 2008	yes	yes	yes	yes	no	yes	no	yes	yes	yes	no	yes	9
An et al.[43] 2020	yes	yes	yes	yes	no	yes	no	yes	yes	yes	no	yes	9
Hsu et al.[44] 2005	yes	yes	yes	yes	no	yes	no	yes	yes	yes	yes	yes	10
Cho et al.[45] 2019	yes	yes	yes	yes	no	yes	no	yes	yes	yes	no	yes	9

regarding the duration of the patients' exposure to OSA, and only 2 studies had the HMP assessor blinded to the severity of OSA.[41,44] Only one study provided a clear justification for the sample size.[40] The main confounders identified in most of the included studies were AHI, BMI and age.

Participants

The study examined data from 7 studies on OSA in 718 adult patients. The average age of the patients was 49.0 ±5.9 years. Out of all OSA patients, 515 (72%) were males with an average age of 47.5 ±4.8 years, while 203 (28%) were females with an average age of 53.0 ±6.7 years. The study noted a significant age difference between men and women ($p < 0.00005$), but there was no significant difference in BMI ($p = 0.9771$) (Table 3). These factors were considered in the analysis of the main outcome measure.

Main findings

As shown in Table 3, the average AHI value was 37.5 ±10.3, with a significant difference between males (37.7 ±10.3) and females (30.5 ±10.3) ($p < 0.00001$). For HMP, the mean value was 20.5 ±3.8 mm, with a significant difference between males (21.6 ±3.3 mm) and females (17.8 ±3.7 mm) ($p < 0.00001$), as shown in Fig. 3. These results stayed very similar after neutralizing the effect of age and BMI (Fig. 4).

The results of Spearman's correlation analysis (Table 4) showed a significant moderate positive correlation between HMP and AHI in women ($r = 0.423$; $p < 0.00001$). However, the correlation was weak and positive in men ($r = 0.167$; $p < 0.00001$). Additionally, a significant weak positive correlation was observed between HMP and BMI in both females ($r = 0.219$; $p < 0.01$) and males ($r = 0.328$; $p < 0.001$). A significant moderate positive correlation was found between BMI and AHI in females ($r = 0.568$; $p < 0.0001$), while a weak positive correlation was observed in males ($r = 0.304$; $p < 0.0001$).

Table 3. ANOVA report summary

Sex	Statistics	HMP [mm]	AHI	BMI [kg/m^2]	Age [years]
Males ($N = 515$)	*M* ±*SD*	21.6 ±3.3	37.7 ±10.3	27.6 ±2.8	47.5 ±4.8
	Me	21.2	36.5	25.7	46.4
	SEM	0.1	0.4	0.1	0.2
	95% *CI* [lower, upper]	[21.3, 21.9]	[36.8, 38.6]	[27.3, 27.8]	[47.0, 47.9]
Females ($N = 203$)	*M* ±*SD*	17.8 ±3.7	30.5 ±10.3	27.6 ±2.0	53.0 ±6.7
	Me	16.3	31.1	27.5	51.7
	SEM	0.2	0.7	0.1	0.4
	95% *CI* [lower, upper]	[17.3, 18.3]	[29.1, 3.9]	[27.3, 27.8]	[52.1, 54.0]
Total ($N = 718$)	*M* ±*SD*	20.5 ±3.8	35.7 ±10.3	27.6 ±2.6	49.0 ±5.9
	Me	20.8	36.5	26.0	47.2
	SEM	0.1	0.4	0.0	0.2
	95% *CI* [lower, upper]	[20.2, 20.8]	[34.9, 36.5]	[27.4, 27.8]	[48.6, 49.5]
p-value		0.0000*	0.0000*	0.9771	0.0000*

BMI – body mass index; *M* – mean; *SD* – standard deviation; *Me* – median; *SEM* – standard error of the mean; *CI* – confidence interval; * statistically significant.

Table 4. Spearman's correlation analysis

Variable		Males ($N = 515$)			Females ($N = 203$)		
		HMP	AHI	BMI	HMP	AHI	BMI
HMP	*r*	1	0.167	0.328	1	0.423	0.219
	p-value	–	0.000*	0.000*	–	0.000*	0.002*
AHI	*r*	0.167	1	0.304	0.423	1	0.568
	p-value	0.000*	–	0.000*	0.000*	–	0.000*
BMI	*r*	0.328	0.304	1	0.219	0.568	1
	p-value	0.000*	0.000*	–	0.002*	0.000*	–

r – Spearman's correlation coefficient; * statistically significant (two-tailed test).

POWERscore	Total			Male			Female			Mean difference
Study	*M*	*SD*	*N*	*M*	*SD*	*n*	*M*	*SD*	*n*	95% *CI*
Chang and Shiao 2008	20.794	9.799	99	21.400	10.030	84	17.400	8.000	15	3.394 [3.198, 3.590]
Hsu et al. 2005	17.801	4.803	65	18.200	4.590	57	14.960	5.870	8	2.841 [2.694, 2.988]
An et al. 2020	19.390	6.258	89	21.270	6.130	55	16.350	6.370	34	3.040 [2.901, 3.180]
Amitani et al. 2020A	26.905	7.473	37	29.500	7.500	21	23.500	7.200	16	3.405 [2.999, 3.812]
Borges et al. 2015	19.277	7.721	102	22.330	8.030	57	15.410	7.220	45	3.867 [3.717, 4.017]
Cho et al. 2019	18.052	6.527	158	19.100	6.400	135	11.900	7.100	23	6.152 [6.070, 6.233]
Amitani et al. 2020B	26.353	5.576	75	30.300	5.800	35	22.900	5.300	40	3.453 [3.306, 3.601]
Tuna et al. 2012C	23.637	1.675	34	24.370	1.230	27	20.810	2.720	7	2.827 [2.728, 2.927]
Tuna et al. 2012B	19.397	1.451	35	20.320	1.310	26	16.730	1.730	9	2.667 [2.583, 2.750]
Tuna et al. 2012A	22.023	1.287	24	22.990	1.210	18	19.120	1.400	6	2.903 [2.793, 3.012]
Total	**20.533**	**6.671**	**718**	**21.624**	**6.746**	**515**	**17.767**	**6.357**	**203**	**2.766 [2.748, 2.785]**

Fig. 3. Meta-analysis of the hyoid bone–mandibular plane distance (HMP) values with regard to sex

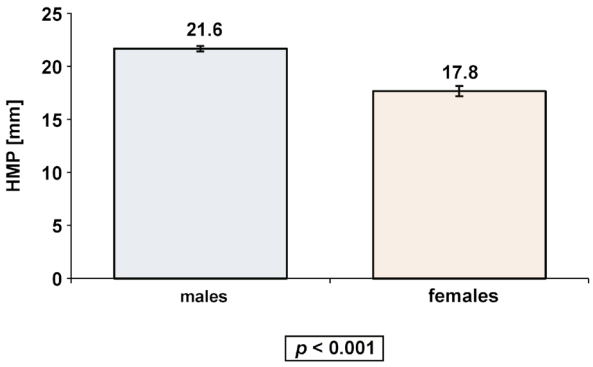

Fig. 4. Estimated marginal mean values of the hyoid bone–mandibular plane distance (HMP) for both sexes

Table 5 presents findings from the linear regression analysis investigating the influence of sex on HMP, independent of age, BMI and AHI, and the correlation between sex and AHI. The analysis indicates a significant impact of sex on HMP. No significant relationship was observed beyond the main effect of sex and OSA.

Confidence in cumulative evidence

According to the GRADE guidelines,[37] high-quality evidence supports the observation that there are sex differences in the HMP of patients with OSA (95% confidence interval (*CI*): 2.75, 2.79; $p < 0.0001$; GRADE tool: high-evidence profile). Additionally, there appears to be a stronger correlation between HMP and AHI in females with OSA than in males with OSA. Heterogeneity was calculated using the I^2 statistic, with I^2 values of 30–50% indicating moderate heterogeneity, 51–75% suggesting substantial heterogeneity, and >75% indicating considerable heterogeneity.

Discussion

This is the first comprehensive review and meta-analysis to explore sex-related differences in the hyoid bone position among patients with OSA.

Table 5. Summary of the linear regression analysis

Parameter	B	*SE*	95% Wald *CI*		Hypothesis test			Exp(B)	95% Wald *CI* for Exp(B)	
			lower	upper	Wald χ^2	df	*p*-value		lower	upper
(Intercept)	–4.442	1.9214	–8.208	–0.676	5.345	1	**0.0208**	0.012	0.000	0.509
[SEX=1]	4.573	0.8239	2.958	6.188	30.809	1	**0.0000**	96.859	19.267	486.929
[SEX=0]	0[a]	–	–	–	–	–	–	1	–	–
AHI	0.141	0.0203	0.102	0.181	48.320	1	**0.0000**	1.152	1.107	1.199
[SEX=1] * AHI	–0.013	0.0238	–0.059	0.034	0.279	1	0.5972	0.988	0.943	1.035
[SEX=0] * AHI	0[a]	–	–	–	–	–	–	1	–	–
BMI	0.214	0.0451	0.126	0.303	22.589	1	**0.0000**	1.239	1.134	1.354
Age	0.226	0.0213	0.184	0.268	112.721	1	**0.0000**	1.253	1.202	1.307
(Scale)	8.236[b]	0.4347	7.427	9.134	–	–	–	–	–	–

B – linear regression coefficient; *SE* – standard error; df – degrees of freedom; Exp(B) – odds ratio; [a] set to zero, as the parameter is redundant; [b] maximum likelihood estimate; *p*-values in bold indicate statistical significance.

After conducting a comprehensive review of the literature on the airways, OSA and the hyoid bone, we chose the variable HMP as the focus of our meta-analysis, as among the various variables assessed via cephalometric analysis, HMP consistently exhibited a strong correlation with OSA across multiple studies.[39–45] Consequently, we decided to base our study on this specific variable.

The presented results highlight several key findings regarding the relationship between HMP, AHI, BMI, and sex. Firstly, the data reveals significant differences between males and females in the AHI and HMP values. Males exhibited a higher average AHI (37.7 ±10.3) as compared to females (30.5 ±10.3), indicating a more severe degree of OSA in men. Similarly, the mean HMP value was higher in males (21.6 ±3.3 mm) than in females (17.8 ±3.7 mm). These differences persisted even after controlling for age and BMI.

The correlation analysis further elucidated the relationships between these variables. In women, a significant moderate positive correlation (r = 0.423) was observed between HMP and AHI, suggesting that increased HMP values are associated with higher AHI scores, indicative of more severe OSA. However, this correlation was weaker in men (r = 0.167). Additionally, both sexes exhibited a significant but weak positive correlation between HMP and BMI. The body mass index demonstrated a moderate positive correlation with AHI in females (r = 0.568), but a weaker correlation in males (r = 0.304). This finding highlights the potential influence of obesity on OSA severity, particularly in women.

Obesity is thought to contribute to OSA through several mechanisms.[46] First, excess fat deposits can accumulate in the airways, narrowing the airway passages. Additionally, obesity can lead to diastolic dysfunction and fat accumulation in the diaphragm muscle, obstructing normal breathing. Moreover, in obese individuals, fat tends to build up in the neck region, resulting in a shorter, thicker neck with a smaller, softer upper airway. This makes the upper airway more prone to collapse or close during sleep, increasing the risk of developing OSA.[47] Women have relatively thinner necks on average.[39] The dorsal positional migration of the hyoid inside the female neck due to the deposited fat could have a greater effect on the airway pathways, potentially impacting the severity of OSA in females (expressed in AHI) as compared to males, indicating that there may be sex-based clinical relevance to consider. This hypothesis is supported by a better response of females with OSA to a mandibular advancement device (MAD) in comparison with males, especially in severe OSA, but also across a range of AHI thresholds.[47] The potential mechanisms underlying the differences between sexes may also be related to the different morphology of the hyoid bone in women and men. In men, the hyoid angle is greater; with advancing age, the hyoid bone moves posteriorly, in rotation, and its position lowers.[48] The weakening of the muscles with age may also affect OSA morbidity. Additionally, anatomical differences in HMP between sexes is likely influenced by the complex interplay of sex hormones, developmental processes and aging. While men tend to have a longer upper airway, predisposing them to a higher OSA risk, women benefit from protective mechanisms beyond simple anatomical differences. These mechanisms involve hormonal influences, differences in tissue properties and potentially more efficient neuromuscular responses in the upper airway.[48–51]

In a recent systematic review and meta-analysis conducted by Camañes-Gonzalvo et al., focusing on identifying the phenotypic characteristics of responders to oral devices (MAD), it was found that responders, as compared to non-responders, are younger patients with a smaller neck circumference, a lower BMI and a shorter distance from the hyoid bone to the C3,[52] which points to the need to take into account, among other factors, the hyoid bone position while considering OSA treatment options.

The linear regression analysis revealed a significant impact of sex on HMP, independent of age, BMI and AHI, and the relationship between sex and AHI. This suggests that biological differences between males and females may contribute to variations in HMP, potentially influencing the risk and severity of OSA.

It is worth noting that while lateral CEPH can provide a basic assessment of the hyoid bone position, MRI offers a more comprehensive and detailed evaluation, enabling a better understanding of the role of the hyoid bone position in the pathogenesis of OSA. Dynamic sleep MRI shows the exact sites and pattern of obstruction while asleep, with no radiation as in the case of CEPH.[22] This information can guide treatment decisions, such as implementing surgical interventions (e.g., hyoid suspension or repositioning), or the selection of appropriate oral appliances or myofunctional therapy for managing OSA in specific patients.

Future directions and clinical implications

To ensure the accuracy of the findings of this meta-analysis, it is crucial to establish a standard range of the hyoid bone position (measured through HMP) for healthy adults and elderly individuals, considering sex differences. Furthermore, it is essential to investigate the underlying causes of the downward and backward movement of the hyoid bone in patients with OSA, emphasizing sex-based factors. Such research could lead to a gender-specific approach to treating OSA patients in the future.

Taking both sex and HMP into consideration could potentially serve as a predictor of OSA severity or the treatment response. Clinicians could use this information to evaluate patients' risk, guide treatment decisions or predict the outcomes of interventions, like the application of MAD. Due to anatomical differences between sexes,

women typically require less mandibular advancement than men to achieve the same therapeutic outcome. This suggests that female patients tend to respond more effectively to treatment with smaller degrees of jaw protrusion. Additionally, knowing the position of the patient's hyoid, myofunctional therapists can design personalized exercise programs. For instance, patients with a lower hyoid position might require more focus on exercises that target the suprahyoid muscles. This can help myofunctional therapists establish a baseline and set specific goals for therapy. Regarding surgical planning, HMP might be a predictor of surgical success, particularly for the procedures aimed at increasing the posterior airway space. The measurement of HMP can inform decisions about the type and extent of surgical interventions. For example, it may influence choices between the mandibular advancement, genioglossus advancement or hyoid suspension procedures. Additionally, understanding sex differences in HMP in OSA patients provides a common reference point for discussions between dentists, ear, nose and throat specialists (ENTs), physical therapists, sleep medicine specialists, and other healthcare providers. Finally, a comprehensive grasp of the intricate pathophysiology of OSA paves the way for significant advancement in treatment. First, it enables the creation of therapies tailored to specific pathophysiological endotypes. Second, it propels the field toward precise sex-oriented medicine, potentially offering patients an alternative to the standard continuous positive airway pressure (CPAP) therapy.

Limitations

A standard range of hyoid bone position (measured through HMP) for healthy adults and elderly individuals, considering sex differences, is currently lacking. The reliance of the study on two-dimensional (2D) imaging techniques, such as lateral cephalometry, for measuring HMP is a limitation, as three-dimensional (3D) imaging methods, like MRI, may provide more accurate and comprehensive assessments of the role of the hyoid bone position in the obstruction of the upper airway.

Conclusions

The results of this study underscore the complex interplay between anatomical factors, such as HMP, and physiological variables, including AHI and BMI, in the context of OSA. Furthermore, the observed sex differences highlight the importance of considering gender-specific factors in evaluating and managing OSA. To ensure the accuracy of the findings of this meta-analysis, it is crucial to establish a standard range of hyoid bone position (measured through HMP) for healthy adults and elderly individuals, considering sex differences. In our review, only two studies reported HMP in healthy individuals in women (12.43 ±8.79 mm) and men (16.69 ±4.54 mm).[44,45] The lack of the healthy individual HMP standard reference emphasizes the need for additional studies that would investigate the HMP value among the healthy population. Based on the GRADE guidelines, high-quality evidence supports the significant role of sex with regard to HMP in OSA patients, with a higher correlation in females.

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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Review

Exploring the link between temporomandibular disorders and infectious diseases: A systematic review of comorbidities and underlying mechanisms

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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

Temporomandibular disorders (TMD) are often linked with a variety of comorbidities, which can complicate their diagnosis and management. A systematic literature review (SLR) was conducted to investigate the association between the occurrence of TMD signs and symptoms in patients with infectious diseases.

The present SLR was carried out in PubMed®. The eligibility criteria were established to include patients presenting with TMD signs and symptoms associated with an infection. The search identified 258 records, of which 27, involving 20,489 patients, were included in the qualitative analysis. Three types of associations were identified between the onset of TMD signs and symptoms and the type of infection. The first association is TMD arising from hematogenous spread of the pathogen to the temporomandibular joint (TMJ), the predominant symptoms of which are related to impaired TMJ function. The infection varies in severity and is occasionally asymptomatic, making it challenging to establish a clear connection between pathogen spread and symptoms in the temporomandibular region. Second, TMD resulting from the local spread of pathogens to adjacent tissues within the temporomandibular area were examined. This category included odontogenic infections, upper respiratory tract infections and otogenic infections. Thirdly, TMD associated with chronic systemic infection without arthritis were analyzed, which develop as a consequence of systemic changes due to prolonged illness and/or psychological disorders arising from limited treatment options.

The relationship between the onset of TMD and infectious diseases is complex and multifaceted. A careful differential diagnosis is essential, as TMD can mask an underlying infection, leading to delays in accurate diagnosis and timely anti-infective treatment.

Keywords: rheumatoid arthritis, inflammation, periodontitis

Highlights

- Three primary mechanisms link infectious diseases and temporomandibular disorders (TMD): hematogenous spread to the temporomandibular joint; local spread from adjacent structures; and systemic infections inducing secondary functional or psychosomatic effects.
- Infection-related TMD can clinically resemble primary TMD, presenting with non-specific symptoms such as pain, swelling, trismus, and restricted jaw movement.
- Accurate differential diagnosis is essential, as failure to identify an infectious origin may result in inappropriate management and delayed anti-infective treatment.
- An interdisciplinary diagnostic approach enhances accuracy and supports optimal treatment outcomes in patients with TMD symptoms potentially associated with infection.

Introduction

Temporomandibular disorders (TMD) are often associated with a range of comorbidities, which can complicate their diagnosis and management.[1,2] Recognizing the potential connections between TMD and other conditions is crucial in clinical practice, as the presence of one disorder may indicate an elevated risk of developing another. This association highlights the importance of a comprehensive approach to patient assessment, where clinicians remain vigilant for signs of related conditions. Overlapping symptoms between TMD and their comorbidities can complicate an accurate diagnosis, making careful differentiation essential to avoid misdiagnosis and establish effective treatment strategies. The presence of less common associations between conditions may result in missed diagnoses, potentially delaying necessary treatment if not thoroughly investigated. Being aware of these links enables a more holistic and effective approach to patient care.

Reports from the literature show that patients with chronic diseases are more prone to develop TMD. A systematic literature review (SLR) conducted by Hysa et al., which included 56 studies, reported a prevalence of TMD in rheumatoid arthritis ranging from 8% to 70%.[3] Identified risk factors for the development of TMD in patients with rheumatoid arthritis included female sex, younger age, positivity for anti-citrulline peptide autoantibodies, higher disease activity, cervical spine involvement, and the presence of cardiovascular and neuropsychiatric comorbidities. Similarly, TMD symptoms, including pain, tenderness upon palpation of the temporomandibular joint (TMJ) and masticatory muscles, joint noises (e.g., clicking or crepitus), limited mouth opening, disc displacement, and radiographic changes, are prevalent in spondyloarthritis, with a reported prevalence ranging from 12% to 80%.[3,4] A systematic review and meta-analysis conducted by de Oliveira-Souza et al. examined the association between TMD and cervical musculoskeletal disorders.[5] The study found that individuals with TMD have reduced endurance of the neck extensors, global and upper cervical hypomobility, and report greater neck disability compared to those without TMD signs and symptoms.[5] Silva et al., in a systematic review and meta-analysis, found that the prevalence of degenerative disease in TMD patients with disc displacement is approx. 50%.[6] A higher prevalence of the condition was identified in individuals with disc displacement without reduction, at a rate of 66%, compared to 35% in those with disc displacement with reduction.[6] Sclerosis and erosion were identified as the most common radiological signs associated with the progression of degenerative joint disease. Clinicians have noted additional associations between TMD and musculoskeletal disorders in daily practice. However, findings from broader analyses remain inconclusive. In particular, the correlations between TMD and generalized joint hypermobility, TMD-related pain following whiplash trauma, and comorbidity with chronic fatigue syndrome have shown inconclusive results, primarily due to insufficient high-quality evidence supporting these relationships.[7–12]

The association between TMD and musculoskeletal disorders is largely due to shared biomechanical stress and inflammatory mechanisms that commonly affect joints and connective tissues throughout the body. In contrast, the higher prevalence of TMD in individuals with conditions affecting other body systems is not as well understood. In clinical practice, it has been observed that patients with infectious diseases, especially those with limited treatment options or complicated by a chronic or severe course, frequently report signs and symptoms of TMD. To shed further light on this connection, we conducted an SLR to investigate the association between the occurrence of TMD signs and symptoms in patients with infectious diseases. An additional aim of the review was to explore the variety of pathomechanisms and infection types that co-occur with TMD signs and symptoms or directly lead to their development.

Material and methods

Literature search and eligibility criteria

This SLR was conducted in PubMed® and supplemented by a Google search of gray literature, following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement.[13]

Additionally, references from the identified review articles were investigated to identify relevant articles that were not included in the PubMed® search. The searches were performed on November 2, 2024. The eligibility criteria were defined according to the Population, Intervention, Comparison, Outcomes, and Study design (PICOS) framework,[14] as shown in Table 1. No restrictions were applied to the timeframe or geographical scope. The SLR aimed to include articles written in English.

The eligibility criteria were established to include patients presenting with TMD signs and symptoms associated with an infection. A strict definition of TMD based on the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD)[15] was not required, allowing for a broader range of infectious diseases to be considered, in which TMD should be evaluated in the differential diagnosis, including cases outside of dental practice.[16]

To assess the quality of the included studies, a set of critical appraisal tools developed by the Joanna Briggs Institute (JBI) was used to assess the methodological rigor of each study.[17] This set of checklists was used to encompass all study designs intended to be included in this comprehensive review.

Search strategy

The search string was developed after establishing the eligibility criteria. Two reviewers participated in screening the titles, abstracts and full texts of the identified records. Discrepancies were resolved by a third reviewer. A similar approach was used for data extraction. The data was collected and extracted using a standardized approach with templates in Microsoft Excel (Microsoft Corp., Redmond, USA). The electronic search strategy, based on relevant keywords related to TMD signs and symptoms in individuals with infectious diseases, is detailed in Table 2.

Table 2. Search strategy

Category	No.	Strategy	Results, *n*
TMD	#1	temporomandibular joint[tw]	32,229
	#2	temporomandibular disorder[tw]	2,339
	#3	temporomandibular joint disorders[tw]	16,062
	#4	temporomandibular joint disease[tw]	162
	#5	temporomandibular joint dysfunction syndrome[tw]	5,050
	#6	((temporomandibular) OR (temporo mandibular[tw])) OR (temporo-mandibular)	34,635
	#7	(TMJ OR TMD)	44,193
	#8	masticatory muscle*[tw]	10,152
	#9	masseter muscle*[tw]	7,120
	#10	(temporomandibular) AND (arthralgia)	734
	#11	masticatory muscle pain[tw]	183
	#12	myofacial pain[tw]	120
	#13	from #1 to #12 with OR	56,326
Infectious diseases	#14	infectious diseases[MeSH Terms]	586,836
	#15	contagious disease[tw]	2,102
	#16	communicable disease[tw]	39,179
	#17	infection OR infectious	4,511,415
	#18	sepsis	226,434
	#19	systemic infection[tw]	7,384
	#20	infectious disease*[tw]	168,941
	#21	viral infection*[tw]	72,901
	#22	bacterial infection*[tw]	147,852
	#23	fungal infection*[tw]	32,471
	#24	parasitic infection*[tw]	11,152
	#25	from #14 to #24 with OR	1,063,326
	#26	#13 AND #25	333
	#27	english[Filter]	317
	#28	humans[Filter]	258

The strategy was implemented in PubMed® on November 4, 2024.
tw – textword; TMJ – temporomandibular joint.

Results

Included studies

The search yielded 258 records, of which 27 were selected for the qualitative analysis.[1,18–43] The process of including identified records is shown in Fig. 1, while the list of the identified studies is presented in Table 3.

Table 1. Eligibility criteria according to the Population, Intervention, Comparison, Outcomes, and Study design (PICOS) framework

Framework	Inclusion criteria	Exclusion criteria
Population	patients presenting signs and symptoms of TMD with or without the diagnosis with DC/TMD; comorbid confirmed or suspected infectious disease	–
Intervention	treated or untreated TMD; treated or untreated chronic/systemic disease	–
Comparison	none or any in comparative studies	–
Outcomes	prevalence/incidence/percentage of the comorbidity in the population of TMD patients	–
Study design	retrospective/prospective, observational, cross-sectional, comparative, and case studies	registries, reviews, editorials, opinions

DC/TMD – Diagnostic Criteria for Temporomandibular Disorders; TMD – temporomandibular disorders.

Table 3. Characteristics of the studies included in the review

Study	Study type	Disease/pathogen	Number of patients/TMD symptoms
Adachi et al.[18] 2000	cross-sectional	tuberculosis *Mycobacterium tuberculosis*	*n* = 17 TMJ pain, limited mouth opening
Alexander and Nagy[19] 1973	case report	septic arthritis *Neisseria gonorrhoeae*	*n* = 1 pain involving left TMJ, difficulty in mastication, limited mouth opening
Ângelo et al.[20] 2023	case report	septic arthritis *Pseudomonas aeruginosa*	*n* = 1 tenderness in the right preauricular area, limited mouth opening, TMJ effusion
Aspesberro et al.[21] 2008	case report	Lemierre's syndrome *Fusobacterium necrophorum*	*n* = 1 swelling in the left retroauricular region, mastoiditis, pain, TMJ effusion
Aufdemorte et al.[22] 1983	case report	myositis *Actinomyces*	*n* = 1 pain, trismus, facial asymmetry, limited mouth opening
Braido et al.[23] 2020	cross-sectional	bronchitis not specified	*n* = 690 DC/TMD symptom questionnaire employed
Castellazzi et al.[24] 2019	case report	otogenic septic arthritis negative blood culture	*n* = 1 torticollis, pain when chewing, progressive trismus, right neck pain, otalgia, swelling over the TMJ, TMJ effusion
Danjou et al.[25] 2022	retrospective cohort	necrotizing external otitis not specified	*n* = 66 symptoms of TMJ arthritis
Døving et al.[26] 2021	case report	Lemierre's syndrome *Streptococcus constellatus*	*n* = 1 pain, trismus, limited mouth opening, swelling and tenderness of the right TMD region, TMJ effusion
Fatima et al.[27] 2020	cross-sectional	Chikungunya disease *Alphavirus chikungunya*	*n* = 253 limited mouth opening, jaw pain
Festa et al.[28] 2022	case report	post-infective osteoarthritis not specified	*n* = 1 pain in the preauricular area, limited mouth opening, severe impairment of the mandibular function, DC/TMD symptom questionnaire employed
Fiorentino et al.[29] 2009	case report	AIDS HIV	*n* = 1 chewing impairment, TMD pain, restricted mouth opening, TMJ clicking during jaw movements
Gherlone et al.[30] 2021	cross-sectional	COVID-19 SARS-CoV-2	*n* = 122 salivary gland ectasia, TMJ abnormalities, facial pain, masticatory muscle weakness
Henry et al.[31] 2001	cross-sectional	sexually transmitted disease *Chlamydia trachomatis*	*n* = 41 salivary gland ectasia, TMJ dysfunction, internal derangement of the TMJ
Jeon et al.[32] 2005	cross-sectional	pharyngitis not specified	*n* = 417 limited mouth opening, noise in the TMJ, pain, jaw stiffness in the morning, DC/TMD symptom questionnaire employed
Klüppel et al.[33] 2012	case report	throat infection *Staphylococcus aureus*	*n* = 1 pain in the left preauricular area, occlusal changes, trismus, limited mouth opening, TMJ effusion
Osiewicz et al.[34] 2019	cross-sectional	Lyme disease *Borrelia burgdorferi*	*n* = 76 DC/TMD symptom questionnaire employed
Osiewicz et al.[35] 2019	cross-sectional	Lyme disease *Borrelia burgdorferi*	*n* = 86 myofascial pain, limited mouth opening, DC/TMD symptom questionnaire employed
Park et al.[36] 2014	case report	tuberculosis *Mycobacterium tuberculosis*	*n* = 1 difficulty in mouth opening, right preauricular painful swelling 3 months later
Park and Auh[1] 2024	cross-sectional	rhinitis not specified	*n* = 2,107 clicking sounds, pain, limited mouth opening, WHO symptom questionnaire employed
Prasad et al.[37] 2007	cross-sectional	tuberculosis *Mycobacterium tuberculosis*	*n* = 165 pain, swelling
Sachs et al.[38] 2020	case report	septic arthritis *Staphylococcus aureus*	*n* = 1 trismus, limited mouth opening, facial pain with tenderness upon palpation of the lateral pole of the TMJ
Santos et al.[39] 2024	cross-sectional	AIDS HIV	*n* = 198 difficulty opening the mouth, muscle fatigue, joint noises, parafunctional habits, DC/TMD symptom questionnaire employed
Simon et al.[40] 2007	cross-sectional	Chikungunya disease *Alphavirus chikungunya*	*n* = 47 TMJ involvement
Staikowsky et al.[41] 2009	cross-sectional	Chikungunya disease *Alphavirus chikungunya*	*n* = 274 TMJ pain
Weise et al.[42] 2021	case report	Lyme disease *Borrelia burgdorferi*	*n* = 1 pain in the right TMJ, limited mouth opening, bruxism, cracking sounds
Yoshizawa et al.[43] 2023	case report	odontogenic infection *Streptococcus constellatus*	*n* = 1 pain in the right upper molars, trismus, limited mouth opening

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; HIV – human immunodeficiency virus; AIDS – acquired immunodeficiency syndrome; COVID-19 – coronavirus disease 2019; WHO – World Health Organization; DC/TMD – Diagnostic Criteria for Temporomandibular Disorders.

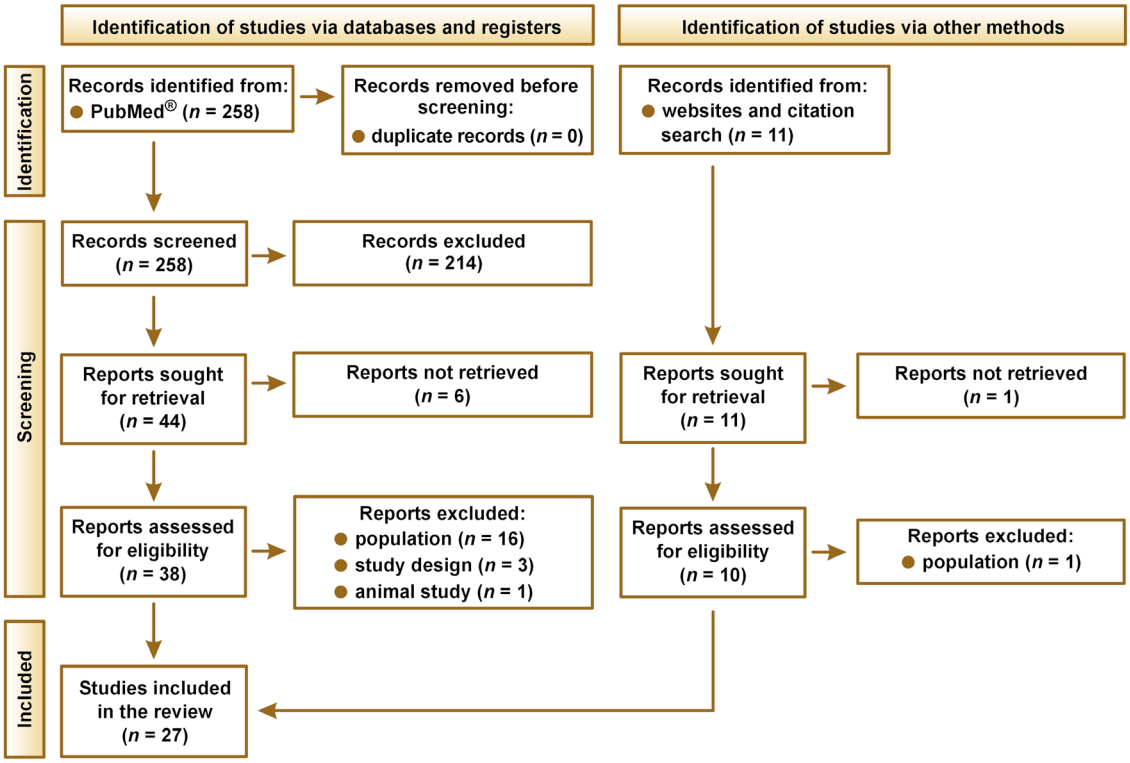

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flow diagram

Summary of the included studies

The SLR included 27 studies involving 20,489 patients. The largest study was a cross-sectional study conducted within the framework of the Korea National Health and Nutrition Examination Survey (KNHANES).[1] The review encompassed 12 cross-sectional studies, 2 cohort studies and 13 case reports.

The qualitative analysis identified 3 types of associations between the onset of TMD signs and symptoms and the type of infection (Fig. 2):

1. TMD signs and symptoms arising from the hematogenous spread of pathogens to the TMJ: predominant symptoms were related to impaired TMJ function. The infection varied in severity and was sometimes asymptomatic, making it challenging to establish a clear connection between the pathogen spread and symptoms in the temporomandibular region;
2. TMD signs and symptoms resulting from the local spread of pathogens to adjacent tissues involving the temporomandibular area: this category includes odontogenic infections in the oral cavity, upper respiratory tract infections and otogenic infections;
3. TMD associated with chronic systemic infection without arthritis: in these cases, TMD signs and symptoms may develop as a consequence of systemic changes due to prolonged illness and/or psychological disorders arising from limited treatment options.

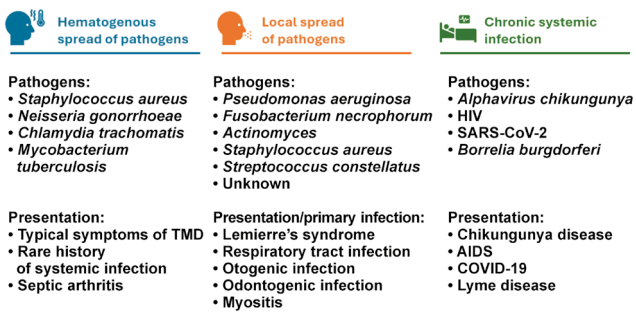

Fig. 2. Three pathways linking the symptoms of temporomandibular disorders (TMD) and infectious diseases

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; HIV – human immunodeficiency virus; AIDS – acquired immunodeficiency syndrome; COVID-19 – coronavirus disease 2019.

Quality assessment

The studies were assessed for trustworthiness, relevance, findings, and the risk of bias (Table 4–6). Among the included case reports, 11 out of 13 studies (85%) received scores of 7–8, while 2 (15%) received scores of 5–6. The most common shortcoming of these studies was the lack of formally articulated takeaway lessons. Only 1 study was classified as a cohort study; however, it was retrospective and lacked a control group. It received 9 out of 11 points. Among the 13 cross-sectional studies, the scores ranged from 4 to 8, with 7 studies (54%) receiving the maximum score of 8. The most common shortcoming in these studies was the failure to identify and analyze potential confounding factors.

Table 4. Assessment of the risk of bias in the included case reports

Question	Alexander[19]	Ângelo[20]	Aspesberro[21]	Aufdemorte[22]	Castellazzi[24]	Døving[26]	Festa[28]	Fiorentino[29]	Klüppel[33]	Park[36]	Sachs[38]	Weise[42]	Yoshizawa[43]
Were patient's demographic characteristics clearly described?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Was the patient's history clearly described and presented as a timeline?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Was the current clinical condition of the patient on presentation clearly described?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Were diagnostic tests or assessment methods and the results clearly described?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Was the intervention(s) or treatment procedure(s) clearly described?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Was the post-intervention clinical condition clearly described?	yes	yes	yes	yes	yes	yes	yes	no	yes	yes	yes	yes	yes
Were adverse events (harms) or unanticipated events identified and described?	no	yes	yes	yes	yes	yes	yes	no	yes	yes	yes	yes	yes
Does the case report provide takeaway lessons?	no	no	no	no	no	no	no	no	no	no	yes	yes	yes
Total score	6	7	7	7	7	7	7	5	7	7	8	8	8

Table 5. Assessment of the risk of bias in the included cohort study

Question	Danjou[25]
Were the two groups similar and recruited from the same population?	NA
Were the exposures measured similarly to assign people to both exposed and unexposed groups?	NA
Was the exposure measured in a valid and reliable way?	yes
Were confounding factors identified?	yes
Were strategies to deal with confounding factors stated?	yes
Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	yes
Were the outcomes measured in a valid and reliable way?	yes
Was the follow-up time reported and sufficient to be long enough for outcomes to occur?	yes
Was follow-up complete, and if not, were the reasons to loss to follow-up described and explored?	yes
Were strategies to address incomplete follow-up utilized?	yes
Was appropriate statistical analysis used?	yes
Total score	9

NA – not applicable.

Table 6. Assessment of the risk of bias in the included cross-sectional studies

Question	Adachi[18]	Braido[23]	Fatima[27]	Gherlone[30]	Henry[31]	Jeon[32]	Osiewicz[34]	Osiewicz[35]	Park[1]	Prasad[37]	Santos[39]	Simon[40]	Staikowsky[41]
Were the criteria for inclusion in the sample clearly defined?	yes	yes	no	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Were the study subjects and the setting described in detail?	yes	yes	no	yes	no	yes	yes	yes	yes	yes	yes	yes	yes
Was the exposure measured in a valid and reliable way?	NA	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Were objective, standard criteria used for measurement of the condition?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Were confounding factors identified?	no	yes	no	yes	no	yes	yes	no	yes	no	yes	no	yes
Were strategies to deal with confounding factors stated?	no	yes	no	yes	no	yes	yes	no	yes	no	yes	no	yes
Were the outcomes measured in a valid and reliable way?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Was appropriate statistical analysis used?	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Total score	5	8	4	8	5	8	8	6	8	6	8	6	8

Septic bloodborne temporomandibular arthritis

The TMJ may be the only joint affected in certain bacterial, viral or fungal infections, complicating the differential diagnosis. The infectious agent typically reaches the TMJ via hematogenous spread following a systemic infection or injury.[18,19,31,36–38] Patients may present with symptoms localized exclusively in the temporomandibular region, though some may experience mild symptoms in other joints as well. Due to the TMJ-specific nature of these symptoms, patients are often initially referred to dental clinics for the management of TMD.

Alexander and Nagy reported a case of gonococcal arthritis in a patient who presented with symptoms resembling TMD, including pain in the masseter muscles, muscle spasms and limited mouth opening.[19] The patient received symptomatic treatment for 1 month before the correct diagnosis was made.[19]

Henry et al. observed an increased frequency of serum antibodies to *Chlamydia trachomatis* in patients with internal derangement of the TMJ.[31] Among those with positive serology, 86% reported urinary or genital symptoms, though only 14% had a history of sexually transmitted disease, and none had been diagnosed with *C. trachomatis* infection. The study suggests that serologic testing for antibodies to bacteria associated with reactive arthritis could be valuable in evaluating patients with the internal derangement of the TMJ.[31]

Tuberculosis, a systemic infection that can affect the cervical lymph nodes via hematogenous or lymphatic spread from a pulmonary source, can also be associated with TMD pathology, even in the absence of active pulmonary disease.[18] Prasad et al. noted that while TMJ involvement is rare, they identified only 1 case of TMJ tuberculosis among 165 cases of head and neck tuberculosis.[37] Park et al. confirmed that TMJ tuberculosis is difficult to diagnose, presenting a case of a patient who was treated at 2 dental clinics for TMD and osteoarthritis before receiving an accurate diagnosis.[36]

TMD associated with infections of adjacent tissues

The local spread of pathogens to the temporomandibular region predominantly occurs in immunosuppressed patients, elderly individuals, young children, and those with other chronic systemic diseases, who may be more susceptible to atypical infections. The most common sites of primary infection include the oral cavity, ears and upper respiratory tract.

Ângelo et al. presented a case of a patient with diabetes mellitus and a history of liver transplantation who reported pain and swelling of the TMJ, malocclusion, and erythema in the TMJ area.[20] The diagnosis was chronic suppurative otitis media, with a biopsy confirming the presence of *Pseudomonas aeruginosa*. Antibiotic sensitivity testing enabled targeted antibiotic therapy, helping to prevent recurrence. Castellazzi et al. reported a case of otogenic septic arthritis in which the patient developed septic arthritis of the right TMJ, with early involvement of the mandibular bone secondary to acute otitis media.[24] The patient presented with torticollis, trismus, and right preauricular swelling over the TMJ, and was successfully treated with antibiotics alone.[24] Danjou et al. observed that TMJ arthritis can also develop as a complication of necrotizing external otitis, an infection of the skull base typically affecting elderly individuals or those with diabetes.[25] Among patients with necrotizing external otitis, 17% developed TMJ arthritis, with *P. aeruginosa* being the causative pathogen in most cases.[25] Infections that spread contiguously to adjacent structures do not need to be concurrent with their sequelae, as demonstrated by Festa et al., who reported a case of unilateral postinfective osteoarthritis of the left TMJ with mandibular condyle resorption.[28] A 9-year-old girl was diagnosed, according to DC/TMD criteria, with bilateral myalgia of the masticatory muscles and arthralgia in the left TMJ. These symptoms developed 3 years after the patient had experienced otomastoiditis and periorbital cellulitis.[28]

Lemierre's syndrome is characterized by an oropharyngeal infection with bacteremia and suppurative thrombophlebitis of the cervical veins, often complicated by metastatic septic emboli. Its prevalence decreased after the introduction of antibiotics; however, it can still occur in patients without underlying risk factors. In a reported case by Aspesberro et al., a 5-month-old infant developed acute otitis media after 2 days of fever and rhinitis.[21] The infant's condition rapidly deteriorated, presenting with lethargy, swelling in the left retroauricular region, mastoiditis, and TMJ effusion. A biopsy culture identified *Fusobacterium necrophorum*, allowing for the administration of ceftazidime and metronidazole, to which the bacteria were sensitive.[21] Døving et al. reported a similar case involving a man who experienced sudden pain and a sensation of subluxation in the right temporomandibular region while yawning, followed by progressive swelling and tenderness in the TMJ area.[26] The patient's condition worsened over time, eventually leading to Lemierre's syndrome, characterized by thrombophlebitis of the internal jugular vein and septic emboli in the lungs. The development of Lemierre's syndrome was attributed to the local spread of infection by *Streptococcus constellatus*.[26]

In a cross-sectional study by Braido et al., 690 adolescents aged 12–14 years were evaluated.[23] Painful TMD were identified in 16.2% of the participants and were significantly associated with bronchitis (odds ratio (*OR*) = 2.5; $p = 0.003$). The authors attributed this association to the inflammation in the respiratory system.[23] Jeon et al. found that patients with TMD more frequently experienced infections in nearby anatomical structures, such as maxillary sinusitis, rhinitis, tonsillitis, and

pharyngitis.[32] In particular, pharyngitis and sinusitis in TMD patients may serve as risk factors, potentially triggering TMJ symptoms shortly after these infections develop. Klüppel et al. described a case of a woman who developed TMD symptoms and septic arthritis of the TMJ 2 weeks after a throat infection.[33] The presence of *S. aureus* was revealed through culturing methods.[33] Park and Auh analyzed data from the KNHANES, a cross-sectional study involving 2,107 individuals (11.86% of participants) who reported one or more TMD symptoms.[1] The prevalence of TMD was higher in individuals with rhinitis symptoms, irrespective of their gender and age.[1]

Yoshizawa et al. presented a case of a patient with an odontogenic infection following a pulpectomy, which had been misdiagnosed as TMD due to overlapping symptoms.[43] The patient experienced pain in the temporomandibular region and a restricted mouth opening of 5 mm. The infection ultimately led to life-threatening complications, including meningitis and septic shock.

Eosinophilia-associated myopathies are clinically and pathologically diverse conditions. While some cases may be linked to parasitic or bacterial infections or various systemic disorders, some present with no identifiable etiologic factors and are classified as idiopathic eosinophilic myositis. Aufdemorte et al. presented a case involving a patient with right-sided facial swelling located at the superficial pole of the parotid gland and masseter muscle, accompanied by pain, restricted mouth opening and trismus.[22] The patient also had severe periodontal disease. Microscopic examination of the biopsy revealed eosinophilic myositis of the masseter muscle, and needle aspiration identified numerous filamentous organisms surrounded by neutrophils, consistent with *Actinomyces* species. However, no *Actinomyces* bacteria were isolated from blood cultures. The patient's condition improved following intravenous administration of penicillin.[22]

TMD associated with long-term systemic infections

Chronic infectious diseases are associated with multiple disorders, potentially contributing to wasting of the body and the progression of frailty. Santos et al. investigated a cohort of 198 patients infected with human immunodeficiency virus (HIV), finding a TMD prevalence of 33.8%.[39] The primary symptoms reported were difficulty opening the mouth, muscle fatigue, joint noises, and parafunctional habits. The logistic regression analysis identified an association between TMD and depression (*OR*: 1.045, 95% confidence interval (*CI*): 1.005–1.087), which can partly explain the presence of TMD in this population.[39] Fiorentino et al. highlighted that many patients undergoing antiretroviral therapy report chronic pain and joint pathologies.[29] The authors presented a case of an HIV-infected patient who developed severe TMJ pain 8 to 9 months after starting HIV therapy. Analgesic treatment and physiotherapy proved ineffective, and the authors concluded that symptom relief could be achieved by adjusting the therapy, specifically through the replacement or reduction of protease inhibitors. However, the patient was lost for observation.[29]

Gherlone et al. conducted a study on patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) to investigate the co-occurrence of oral symptoms in coronavirus disease 2019 (COVID-19) survivors.[30] Their findings revealed that 43% of the survivors exhibited salivary gland ectasia, 19% experienced masticatory muscle weakness, 7% had TMJ abnormalities, and 2% reported facial pain. All patients had achieved effective viral clearance, excluding the direct cytopathic effect of the virus as a cause. However, a strong association was identified between salivary gland ectasia and elevated levels of C-reactive protein (CRP), a marker of systemic inflammation, and lactate dehydrogenase (LDH), a marker of tissue necrosis, at the onset of symptoms.[30] These outcomes underscore the critical role of the innate immune response in the oral cavity.

Lyme disease, the most common tick-borne illness, is caused by spirochete bacteria from the *Borrelia* species. The condition can lead to large joint arthritis, disseminated infection, or symptoms indicative of peripheral nerve damage. Cases of Lyme disease presenting solely with TMJ symptoms are rare. Weise et al. reported a case of a 25-year-old woman with severe TMD symptoms who was ultimately diagnosed with Lyme disease after undergoing extensive treatment targeting the TMJ.[42] The prominence of TMD symptoms contributed to a delayed diagnosis by several years. A cross-sectional study conducted by Osiewicz et al. revealed that 70% of 86 adult patients with Lyme disease were positive for an DC/TMD diagnosis of myofascial pain.[35] In another study, Osiewicz et al. compared 76 adult patients with Lyme disease with 54 volunteers without Lyme disease.[34] Patients with Lyme disease were characterized by a higher prevalence of osteoarthrosis (17.1% vs. 5.6%; $p < 0.001$), myogenous pain (18.4% vs. 7.4%; $p = 0.059$), moderate/severe somatization (73.7% vs. 7.4%; $p < 0.001$), and high levels of chronic pain-related impairment (15.8% vs. 0.0%; $p = 0.002$). What is more, the prevalence of depression, ranging from moderate to severe, was found to be significantly higher among patients diagnosed with Lyme disease compared to those without (55.3% vs. 7.4%; $p < 0.001$).

Chikungunya is an infection caused by *Alphavirus chikungunya*. The virus is transmitted to humans by mosquitoes. Given the absence of a definitive treatment, the condition becomes chronic. As reported by Fatima et al., patients suffering from Chikungunya virus presented with difficulty in mouth opening and jaw pain.[27] The prevalence of oral symptoms in these patients was found to be 74%.[27] Simon et al. reported the involvement of the TMJ in 15% of infected patients.[40] Staikowsky et al. noted that symptoms suggesting the involvement of the TMJ were more frequent in patients without viremia in comparison to those with viremia (3.2% vs. 1.8%), though this difference was not statistically significant.[41]

Discussion

Our review indicates that signs and symptoms of TMD are common in patients with infectious diseases. The qualitative analysis identified 3 types of associations between TMD manifestations and the type of infection. In cases where TMD signs and symptoms arise from hematogenous spread of the pathogen to the TMJ, impaired TMJ function predominates. When pathogens spread locally to tissues around the temporomandibular area, the primary infection site may be overlooked. This category includes odontogenic infections in the oral cavity, upper respiratory tract infections and otogenic infections. In patients with chronic systemic infections, TMD may develop due to systemic changes from prolonged illness and/or psychological disorders resulting from limited treatment options. The association between infections and pathologies in the temporomandibular region may not always be immediately apparent, underscoring the importance of a comprehensive evaluation.

The pathogenesis of TMD is not fully understood. Recent research has highlighted the role of elevated inflammatory processes in patients with TMD. Ismah et al. investigated the levels of inflammatory biomarkers in patients who developed TMD after orthodontic treatment.[44] They found that the mean CRP value in TMD patients was overall normal, but it was significantly elevated in individuals with intra-articular TMD in comparison to those with other types of TMD.[44] Similarly, Zwiri et al. examined the effects of different TMD treatment modalities on inflammatory biomarkers.[45] At baseline, the mean CRP level was 2.85 ±1.13, with no substantial changes following treatment. The study revealed no significant impact of treatment modalities on inflammatory biomarker levels.[45] On the other hand, CRP levels are elevated in patients with TMD and inflammatory diseases or infections.[24,41] This finding does not facilitate differentiation between TMD and overlapping inflammatory processes of various origins. Inflammatory biomarkers are influenced to a greater extent by the presence of infection and inflammation than by the occurrence of TMD alone, making them less useful for differential diagnosis. The epidemiology, pathogenesis and consequences of septic arthritis of the TMJ have been thoroughly documented in the literature.[46,47] The diagnosis of this condition is straightforward when infection symptoms are prominent and functional impairment of the joint follows pathogen invasion. However, the diagnosis becomes more challenging when infection symptoms are mild or the patient remains asymptomatic. In such cases, a thorough differential diagnosis is crucial to ensure the accurate and timely identification of the condition and prevent unnecessary delays.[19,31] Another key finding from the literature is the relationship between bacteremia and the presence of specific bacterial species in the synovial fluid of the TMJ. Jeon et al. confirmed that *S. aureus* can cause hematogenous infection of the TMJ, estimating a 55.5% probability of TMJ infection in cases of *S. aureus* bacteremia.[48] This relationship was not observed for other bacteria, such as *Streptococcus mitis* and beta-hemolytic *Streptococcus*.

Severe long-term complications can arise from septic arthritis of the TMJ.[49] Coleman et al. reported 3 cases of TMJ ankylosis secondary to neonatal group B streptococcal sepsis, in which the patients subsequently developed micrognathia and facial deformities.[50] The restricted growth resulting from TMJ ankylosis significantly reduced mouth opening, leading to airway compromise and surgical intervention. Regev et al. reported similar cases in which TMJ ankylosis developed as a consequence of *S. aureus* sepsis.[51] Patients exhibited facial asymmetry with chin deviation to the right, along with mandibular micrognathia and retrognathia. The maximum mouth opening ranged from 2 mm to 15 mm. Sleep apnea contributed to poor sleep quality.[51] The present review also showed that TMJ involvement can appear long after systemic infection, a finding that is particularly important in children.[24]

The association between chronic systemic infections and TMD extends beyond the primary infection and the direct impact of the pathogen on the body. For instance, changes in body composition, such as lipodystrophy in HIV-infected patients, can contribute to TMD-related complications. Paton et al. reported a reduction in superficial facial fat in patients with weight loss, with changes observed in cheek fat, temporal fat, and the compartments of the masseter and temporalis muscles.[52] Scali et al. described anatomical changes in HIV-infected patients resulting from facial lipoatrophy and posterior cheek enlargement, often due to parotid gland and masseter muscle hypertrophy.[53] Da Silva et al. conducted a cross-sectional study comparing the stomatognathic system function between 30 patients infected with HIV subtype 1, who displayed no signs or symptoms of TMD, and 30 healthy individuals.[54] The study showed that the HIV-infected group exhibited relative limitations in masticatory function during chewing. Ultrasound imaging revealed a greater average muscle thickness in the right and left temporal regions at rest and during maximal voluntary contraction. Furthermore, the study noted an increased average thickness in the right and left temporal regions, as well as in the left sternocleidomastoid muscle, when compared to healthy controls. In addition, Umeda et al. observed differences in mandibular condylar bone microarchitecture among individuals living with HIV.[55] Positive HIV status remained significantly associated with increased trabecular thickness, decreased cortical porosity, and increased cortical bone volume fraction.[55]

The chronic nature of the disease has been demonstrated to contribute to the development of mental disorders and somatization in affected patients. A more frequent occurrence of depression was observed in patients infected with HIV and those with Lyme disease.[34,39] Anxiety and depression are often associated with comorbid TMD, adding another layer of connections between those disorders.[56,57] The COVID-19 pandemic highlighted a potential

vicious cycle, where the stress and anxiety of contracting a contagious infection and the fear of transmitting it to others may exacerbate TMD symptoms, given the psychological component of TMD.[58,59] This relationship was well-studied in patients with COVID-19 during the pandemic period.[60] Anxiety and depression may also contribute to the development of TMD in patients experiencing an acute infection. Askim et al. reported that symptoms of anxiety and depression are associated with an increased risk of bloodstream infections.[61] Their study found that severe depression symptoms were linked to a 38% higher risk of bloodstream infection, adjusted for age, sex and education (hazard ratio (*HR*): 1.38, 95% *CI*: 1.10–1.73). Somatization may play a significant role during infectious diseases. Osiewicz et al. observed that patients with Lyme disease exhibited significantly higher rates of moderate to severe somatization (73.7% vs. 7.4%; $p < 0.001$) and widespread muscle sensitization.[34,35] Similarly, Braido et al. found that adolescents with painful TMD reported a greater number of body pain sites in the previous 12 months (4.26 vs. 2.90; $p < 0.001$) and a higher prevalence of systemic diseases (1.48 vs. 1.18; $p = 0.048$) compared to those without painful TMD.[23] A study by Florens et al. found that, among patients with infectious gastroenteritis, higher levels of anxiety and somatization prior to infection were potential risk factors for the development of post-infectious irritable bowel syndrome and persistent abdominal complaints.[62] Seweryn et al. found that patients with TMD experience high levels of somatization which are associated with elevated levels of central sensitization and greater masticatory muscle pain.[63]

The present review on the relationship between TMD and infections has several limitations that should be considered when interpreting the results. A high degree of heterogeneity was observed in the study designs and populations, with case reports comprising the majority of the included studies. To better investigate diagnostic challenges, we included studies with varying diagnostic criteria; however, this approach resulted in an escalation of heterogeneity. A paucity of studies reported psychological factors, which are important due to their potential to mediate or exacerbate TMD symptoms in patients with chronic or acute infections that progress rapidly. Additionally, geographic and demographic limitations should be noted, as some infections occur only across specific regions or demographic groups, which may not be representative of all populations and could limit the global applicability of findings. Addressing these limitations in future research would provide a more comprehensive understanding of the complex relationship between TMD and infections. Future prospective and experimental studies should focus on establishing the factors responsible for the development of TMD symptoms in patients with infectious diseases, assessing underlying pathophysiological mechanisms, and evaluating standardized diagnostic criteria for improved clinical management. The exploration of immune response variability in TMD patients with infections could provide more profound insights into the underlying causative mechanisms of this connection.

Current clinical guidelines for TMD do not adequately address infections as a potential background factor or a co-occurring condition.[64,65] However, they emphasize the importance of a thorough examination, including palpation of the jaw structures, such as the muscles and TMJ, which can aid in detecting local infections or rheumatoid diseases affecting the joint.[15] To enhance differential diagnosis, we propose incorporating a standardized protocol that includes a detailed medical history focusing on recent or chronic infections, along with a physical examination to identify signs of local inflammation. This approach would improve the early recognition and management of infection-related TMD cases. The present study found that only 6 publications explicitly mentioned using the DC/TMD symptom questionnaire for the identification of patients experiencing TMD symptoms related to infection.[23,28,32,34,35,39] Conversely, some patients underwent treatment for TMD over an extended period before receiving an accurate diagnosis and appropriate anti-infective treatment.[36,42,43] The importance of interdisciplinary collaboration between dentists, infectious disease specialists and mental health professionals for the enhancement of the quality of care for patients with pathologies affecting the temporomandibular area is also emphasized.

Conclusions

The present review reveals that the relationship between the onset of TMD symptoms and infectious diseases is complex and multidimensional. The manifestation of TMD symptoms can be attributed to hematogenous and local spread of pathogens. These symptoms can also develop during chronic systemic infections independently of direct pathogen involvement. Careful differential diagnosis is essential, as TMD can mask an underlying infection, leading to delays in accurate diagnosis and timely anti-infective treatment. Such delays may allow uncontrolled pathogens to spread, resulting in long-term health consequences. Conversely, TMD can also develop as an independent disease entity in patients with chronic infections, further emphasizing the critical importance of a thorough differential diagnosis.

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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Review

Correlation between MRI-detected effusion and temporomandibular joint pain: A systematic review

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Abstract

The purpose of this systematic review was to provide a comprehensive assessment of the literature on the relationship between the presence of effusion, as determined by magnetic resonance imaging (MRI), and clinical pain in patients with temporomandibular disorders (TMD). The study was performed in order to answer the following clinical question: "Can MRI-detected temporomandibular joint (TMJ) effusion be considered a marker of clinical pain?"

On June 15, 2024, a systematic literature review was performed in the PubMed® and Scopus databases. The Medical Subject Headings (MeSH) terms used to initiate the search were "temporomandibular joint" AND "MRI". A PICO (Population, Intervention, Comparison, and Outcome) structured reading model was employed to identify and assess articles that evaluated the correlation between TMJ effusion visible on MRI scans and clinical pain reported by patients. The review adhered to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. A total of 539 articles were initially retrieved, of which 14 answered the research question. The review revealed a consistent pattern of results, with 12 out of the 14 articles reporting an association between effusion and pain.

The findings indicate that there is a link between the occurrence of effusion and the experience of pain in individuals diagnosed with TMD.

Keywords: effusion, temporomandibular joint disorder, magnetic resonance, temporomandibular joint pain

Cite as
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Highlights

- Magnetic resonance imaging (MRI)-detected temporomandibular joint (TMJ) effusion is frequently associated with clinical pain, with most studies reporting a positive correlation with TMJ arthralgia.
- The presence and severity of effusion generally parallel pain intensity, particularly in joints with internal derangement, supporting an inflammatory contribution to symptom development.
- Although joint effusion may also be observed in asymptomatic TMJs, painful joints show a substantially higher prevalence, reinforcing its clinical relevance.
- The overall level of evidence remains low to moderate and heterogeneous, limiting meta-analysis and underscoring the need for standardized pain assessment and imaging protocols.
- Clinically, TMJ pain can serve as a useful predictor of effusion, potentially guiding decision-making and reducing unnecessary MRI use.

Introduction

Temporomandibular disorders (TMD) encompass a group of conditions that affect the temporomandibular joint (TMJ), masticatory muscles and associated structures.[1] These disorders manifest clinically as pain, limited jaw movement, joint noises (clicks), and functional impairment.[2] They are associated with substantial patient discomfort and reduced quality of life. Therefore, research is necessary to provide the best possible care to affected individuals. The association between joint disorders and various clinical and radiological signs and symptoms has been extensively documented in medical literature.[3] Intra-articular joint disorders can be linked to clinical conditions, such as disc displacement (with or without reduction) and radiological signs, including sclerosis, erosion, osteophytes, and subcortical cysts.[4]

Magnetic resonance imaging (MRI) is a valuable diagnostic tool for TMJ pathology due to its superior ability to visualize soft tissue conditions compared to other methods.[5] The tool can provide information about the location of the disk,[6] synovial fluid quantity,[7] and the condition of the retrodiscal tissues and bone marrow.[8] The majority of MRI studies has focused on signal changes within joint compartments.[9] These alterations indicate the presence of fluid resulting from the inflammation of retrodiscal tissues and other inflammatory changes in the synovial membrane, which can lead to joint effusion (JE).[10] The clinical assessment of pain and the study of its correlation with MRI findings is a key factor to guide the diagnostic and therapeutic dimensions of TMD management.[7] An enhanced understanding of the connection between the presentation of clinical pain and the imaging features revealed by MRI can greatly influence treatment decisions and help tailor interventions to address the specific needs of individual patients.[11,12]

The aim of this systematic review is to examine the existing literature to address the clinical question of whether effusion observed on MRI can be considered a marker for arthralgia.

Material and methods

Research strategy

A systematic review of the literature published until June 2024 addressing the relationship between the presence of TMJ effusion on MRI and pain reported by patients with TMD was carried out in the PubMed® and Scopus databases. The review process followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[13] The research has been registered with PROSPERO (ID No. CRD42024558402). Studies referenced within the reviewed articles were also included if they met the established inclusion criteria. The database search used a combination of Medical Subject Headings (MeSH) terms as well as expansion strategies based on the evaluation of reference lists of included articles and authors' personal libraries:

– ("temporomandibular joint"[MeSH Terms] OR ("temporomandibular"[All Fields] AND "joint"[All Fields]) OR "temporomandibular joint"[All Fields] OR "tmj"[All Fields]) AND ("magnetic resonance imaging"[MeSH Terms] OR ("magnetic"[All Fields] AND "resonance"[All Fields] AND "imaging"[All Fields]) OR "magnetic resonance imaging"[All Fields] OR "mri"[All Fields]);
– ("magnetic resonance imaging"[MeSH Terms] OR ("magnetic"[All Fields] AND "resonance"[All Fields] AND "imaging"[All Fields]) OR "magnetic resonance imaging"[All Fields] OR "mri"[All Fields]) AND ("temporomandibular joint"[MeSH Terms] OR ("temporomandibular"[All Fields] AND "joint"[All Fields]) OR "temporomandibular joint"[All Fields]) AND ("effusate"[All Fields] OR "effusates"[All Fields] OR "effused"[All Fields] OR "effusion"[All Fields] OR "effusions"[All Fields] OR "effusive"[All Fields]);
– ("magnetic resonance imaging"[MeSH Terms] OR ("magnetic"[All Fields] AND "resonance"[All Fields] AND "imaging"[All Fields]) OR "magnetic resonance imaging"[All Fields] OR "mri"[All Fields]) AND ("temporomandibular joint"[MeSH Terms] OR ("temporomandibular"[All Fields]

AND "joint"[All Fields]) OR "temporomandibular joint"[All Fields]) AND ("edema"[MeSH Terms] OR "edema"[All Fields] OR "edemas"[All Fields] OR "oedemas"[All Fields] OR "oedema"[All Fields]).

Inclusion criteria

The inclusion criteria encompassed clinical trials, cohort studies, case–control studies, and case series that investigated the correlation between MRI effusion in the TMJ and pain, and were published in English. The following types of publications were excluded from the analysis: systematic reviews or meta-analyses; non-systematic reviews; case reports; expert opinions; letters; studies that did not report a correlation between effusion and pain; studies that reported data from previous publications; opinion papers; letters to the editor; and articles published before 1990.

Assessment of papers

The literature screening was carried out using a systematic approach to identify all relevant articles. During the review process, the titles and abstracts were initially screened (TiAb screening), followed by the full-text reading of the papers that passed the filter. The process was carried out by 3 different reviewers (FS, NGS, MV) who worked separately and later discussed their differences. The full texts of the articles that met the eligibility criteria were retrieved and thoroughly reviewed together with the review coordinator (DM). The following data was extracted: author(s); year of publication; study design; sample size; sex and age of participants; follow-up period; outcome variables; and results.

The articles were analyzed by adopting a PICO (Population, Intervention, Comparison, and Outcome) strategy for structured reading, based on the following question: In individuals diagnosed with TMD (P), does the presence of effusion (I), as compared to joints without effusion (C), correlate with the reported pain experienced by the patients (O)? A descriptive analysis was subsequently conducted on the selected studies.

Assessment of study quality

The grading of the level of evidence was based on the work of Sackett, as summarized in Table 1.[14]

Statistical analysis

The substantial heterogeneity of the included studies precluded the possibility of conducting a meta-analysis. Thus, the present systematic review was subjected to a descriptive analysis. EndNote 20 (Clarivate Analytics, London, UK) was employed to organize the reviewed studies, while Microsoft Excel (v. 16.93.1 for Apple; Microsoft Corp., Redmond, USA) was utilized to catalog the results and characteristics of the selected studies.

Table 1. Sackett's levels of evidence[14]

Level	Intervention studies
I	• systematic reviews of RCTs • large RCTs (n > 100, narrow *CIs*)
II	• smaller RCTs (n < 100, wider *CIs*) • systematic reviews of cohort studies • outcomes research (very large ecologic studies)
III	• cohort studies with a concurrent control group • systematic reviews of case–control studies
IV	• case series • cohort studies without a concurrent control group (e.g., with a historical control group) • case–control studies
V	• expert opinions • case studies/reports • bench research • expert opinions based on theory or physiologic research • common sense/anecdotes

RCTs – randomized controlled trials; *CI* – confidence interval.

Results

A total of 539 articles were identified, and 295 papers were excluded after an initial screening of titles. Subsequent analysis of the abstracts of the remaining articles resulted in the exclusion of an additional 188 studies. The final step involved a thorough review of the full text of 56 articles. Of these, 37 articles did not study the variables of interest, and 5 articles assessed a population that was not homogeneous, leading to a total of 14 papers included in the review. The study selection process is illustrated in Fig. 1.

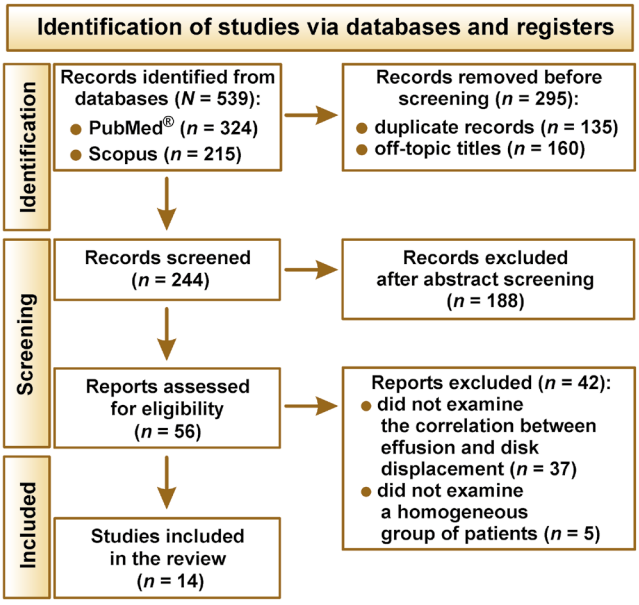

Fig. 1. Flowchart for paper selection

Study characteristics

Table 2 displays the characteristics of the included studies.

Table 2. Characteristics of the included studies

Study	Population	Intervention (MRI machine, MRI analysis, clinical investigation)	Comparison (control group)	Outcome	Correlation between effusion and pain	Follow-up
Westesson and Brooks 1992[16]	390 patients (including 11 healthy controls)	1.5T scanner: axial localizer; sagittal open/closed-mouth sequences, coronal closed-mouth sequences	yes	A strong association was observed between JE observed on MRI and pain ($p < 0.001$).	present	no follow-up
Roh et al. 2012[20]	254 patients (92 M, 162 F), 508 TMJs mean age: 30.5 ±12.0 years	1.5T MRI interpreted by 2 examiners	no	Pain was reported more frequently in the ADDwR and ADDwoR groups compared with the normal group. In these groups, a correlation with effusion was also identified ($p < 0.05$).	present	no follow-up
Matsubara et al. 2018[15]	425 patients (97 M, 328 F), 850 TMJs	1.5T MAGNETOM Vision, 1.5T Achieva, 3.0T MAGNETOM Skyra, or 3.0T MAGNETOM Verio: T1–T2 sagittal open/closed-mouth imaging	no	Significant correlations were identified between pain and JE grades 2 ($p = 0.01$) and 3 ($p = 0.00$).	present	no follow-up
Hosgor 2019[23]	120 patients (17 M, 103 F), included only patients with unilateral, painful temporomandibular disorder with ADDwR and ADDwoR with and without limited opening mean age: 29.9 ±12.0 years	0.5T scanner: T1–T2 sagittal open/closed-mouth and coronal sequences	yes (pain-free side used as control)	A significant relationship was observed between pain and JE ($p < 0.05$).	present	no follow-up
Güler et al. 2005[21]	study group: 3 M and 13 F; control group: 5 M and 10 F mean age: 31 years (study group); 28 years (control group)	1.5T scanner: T1–T2 sagittal open/closed-mouth and coronal sequences	yes	No significant correlation was noted between the level of pain/dysfunction and either effusion or total protein concentration in the control and study groups ($p > 0.05$).	absent	no follow-up
Fernández-Ferro et al. 2023[27]	298 patients (22 M, 276 F) mean age: 38.59 ±12.76 years	1.5T scanner: T1–T2 sagittal open/closed-mouth and axial/coronal sequences	no	A significant relationship was observed between pain and effusion ($p = 0.003$).	present	no follow-up
Díaz Reverand et al. 2020[26]	203 patients (15 M, 188 F) mean age: 40.41 years	1.5T scanner: T1–T2 sagittal open/closed-mouth and coronal closed-mouth sequences	no	A positive relationship was reported between effusion and pain ($p = 0.001$).	present	clinical follow-up at 3, 6 and 12 months post-surgery
Yamamoto et al. 2003[19]	293 patients (62 M, 242 F), 577 TMJs mean age: 31.4 years (range: 10–78)	324 joints: 1.0T MAGNETOM Expert; 124 joints: 1.5T MAGNETOM Vision; 129 joints: 1.5T Signa: T1–T2 sagittal open/closed-mouth and axial sequences	no	In the DDwoR group, pain was significantly correlated with the amount of effusion ($p < 0.001$).	present	no follow-up
Rudisch et al. 2001[18]	41 patients (9 M, 32 F) mean age: 39.1 years (range: 17–78)	1.5T scanner: T1–T2 sagittal open/closed-mouth sequences	no	TMJ pain was correlated with the presence of effusion ($p = 0.004$).	present	no follow-up
Pinto et al. 2021[25]	116 patients (38 M, 78 F)	1.5T scanner; T1–T2 sagittal open/closed-mouth and axial/coronal sequences	no	No association was observed between pain and effusion ($p > 0.05$).	absent	no follow-up
Lee et al. 2009[24]	66 patients (12 M, 54 F), 132 TMJs mean age: 29 years (range: 13–65)	1.5T scanner: T2-weighted sagittal open/closed-mouth imaging	no	A correlation was found between RSI (effusion) and pain ($p < 0.05$).	present	no follow-up
Koca et al. 2020[17]	350 patients (179 M, 171 F), 700 TMJs mean age: 31 years (range: 12–65)	1.5T scanner: T1–T2 sagittal and coronal sequences, both open and closed mouth	no	Pain levels in the JE group were significantly higher than in the non-JE group ($p < 0.05$).	present	no follow-up

Study	Population	Intervention (MRI machine, MRI analysis, clinical investigation)	Comparison (control group)	Outcome	Correlation between effusion and pain	Follow-up
Haley et al. 2001[22]	85 F	1.5T scanner: single radiologist; T1–T2 sagittal open/closed-mouth and coronal closed-mouth imaging	yes (pain-free side used as control)	A significant correlation was identified between the presence of effusion and pain ($p = 0.001$). The likelihood of effusion present on the painful side was 3.8-fold higher.	present	palpation examination and MRI performed 1 week post-procedure
Manfredini et al. 2003[7]	61 patients	0.5T scanner: 2 radiologists; T2-weighted open/closed-mouth imaging	no	A correlation was noted between effusion and pain ($p < 0.05$).	present	no follow-up

M – males; F – females; ADDwR – anterior disc displacement with reduction; ADDwoR – anterior disc displacement without reduction; MRI – magnetic resonance imaging; TMJ – temporomandibular joint; RSI – relative signal intensity; JE – joint effusion.

A wide variation in the composition of the different study groups was identified. The majority of articles included only populations of individuals with unspecific TMJ pain, either bilateral[7,15–20] or unilateral.[21–23] However, 2 studies[21,24] focused exclusively on patients who exhibited symptoms of TMJ pain, TMJ clicks, and limited mandibular opening. One study included only patients with clinical signs and symptoms of disc displacement with reduction (DDwR).[25] Díaz Reverand et al. recruited patients with painful TMJ disease who underwent unilateral arthroscopy and had both preoperative MRI and clinical follow-ups conducted at 3, 6 and 12 months post-surgery.[26] Symptomatic patients with internal derangement who did not respond to conservative treatment were recruited by Fernández-Ferro et al.,[27] while Roh et al.[20] adopted a random selection approach when choosing MRI scans from patients with TMD.

The vast majority of studies employed a 1.5T machine. Exceptions were observed in the studies by Hosgor et al.[23] and Manfredini et al.,[7] which relied on a 0.5T system; Yamamoto et al.,[19] who additionally incorporated a 1.0T scanner; and Matsubara et al.,[15] who also employed a 3.0T machine. A high degree of similarity was observed in the sequencing techniques used in the studies. Almost all studies assessed both T1- and T2-weighted images in closed- and open-mouth positions.

Quality of the selected studies

Table 3 depicts a predominance of low-level (level III/IV) evidence within the included studies.

Effusion and pain

The reviewed papers consistently reported an association between JE, as observed on MRI, and clinical pain, with minor exceptions. Studies by Güler et al.[21] and Pinto et al.[25] did not report any association between JE and pain. Hosgor noted a relationship between marked effusion and pain, but was unable to identify a statistically significant correlation between moderate effusion and pain.[23]

Table 3. Level of evidence of the included studies based on the Sackett's scale

Study	Level of evidence
Westesson and Brooks 1992[16]	III
Roh et al. 2012[20]	IV
Matsubara et al. 2018[15]	IV
Hosgor 2019[23]	III
Güler et al. 2005[21]	III
Fernández-Ferro et al. 2023[27]	IV
Díaz Reverand et al. 2020[26]	IV
Yamamoto et al. 2003[19]	IV
Rudisch et al. 2001[18]	IV
Pinto et al. 2021[25]	IV
Lee et al. 2009[24]	IV
Koca et al. 2020[17]	IV
Haley et al. 2001[22]	IV
Manfredini et al. 2003[7]	IV

Discussion

The attempt to identify a correlation between clinical symptoms and radiological signs on MRI is a highly sensitive topic in the literature. Numerous articles have endeavored to correlate disc displacement and JE.[11,28,29] The potential for a psychologically modulated condition in patients experiencing TMJ pain without signs of effusion was also investigated.[30] On the other hand, the presence of intra-articular fluid, as detected by MRI, remains a subject of debate in relation to its association with TMJ

pain. It has been suggested that a certain amount of TMJ effusion may be present among asymptomatic individuals,[31] but JE is considered a radiological sign of osteoarthritis when accompanied by cortical bone erosion and/or productive bone changes, thus making it worthy to explore as a source of clinical findings.[32] The prevalence of TMJ effusion in patients with TMJ pain ranged from 13% to 88%, whereas prevalence rates in TMJs without pain ranged from 0% to 38.5%.[21,33–36]

The review revealed a statistically significant correlation between JE and pain in 12 of the 14 papers examined.[7,15–20,22–24,26,27] Among the papers reporting a correlation, Hosgor conducted a study on 240 TMJs from 120 patients, noting a statistically significant difference in TMJ pain levels between patients with severe JE and individuals without effusion.[23] Among the negative studies, Güler et al. did not identify a significant correlation between pain and dysfunction levels and JE and total protein concentration, either in control or study groups ($p > 0.05$).[21] However, it is important to note that this study was conducted on only 31 patients. Further research with a larger sample size is necessary to obtain conclusive results. Pinto et al. failed to establish a correlation between pain and JE.[25] However, 71% of the patients exhibited moderate to severe pain (i.e., visual analogue scale (VAS) > 5), indicating the necessity for further data refinement.[25]

The results, as well as the partial heterogeneity of findings, may be related to the non-homogeneous approach to pain diagnosis and clinical evaluation. The subjective nature of pain, influenced by the psychological status of the patient and the different approaches to its diagnosis, may also play a role. It has been observed that certain patients who report pain in the TMJ area in the absence of effusion may be experiencing this discomfort due to a condition that is psychologically modulated.[30] This finding suggests that a careful evaluation of the patient's psychological status might be necessary along with a thorough physical and imaging examination.

The clinical management of TMD represents a significant challenge for clinicians. In this context, the reported agreement between clinically predicted cases of DDwR and disk displacement without reduction (DDwoR) with MRI findings is noteworthy. The findings suggest that a standardized assessment conducted by a trained examiner is useful in evaluating patients with TMD. Nonetheless, the potential for the overdiagnosis of JE, DDwR and DDwoR on MRI in the absence of clinical symptoms highlights the need for further studies.[37]

In the study conducted by Koca et al., which also assessed the intensity of pain, the pain score in the group with JE was significantly higher than in the group without JE ($p < 0.05$).[17] The same study suggested that disks with round shapes were more commonly found in patients without JE ($p < 0.001$), and that folded disk type was more common in patients with JE ($p < 0.001$).[17] Westesson and Brooks found a strong correlation between TMJ pain, disc displacement and JE.[16]

Multiple studies on MRI of the TMJ[11,16,17,38,39] showed that patients with DDwR have a higher prevalence of JE on MRI scans and clinical pain when compared to patients with a normal disk–condyle relationship. Abnormal mechanical loads on joints with a displaced disk may lead to molecular events that generate free radicals and nitric oxide, thus explaining the presence of joint inflammation and pain.[40] Roh et al. demonstrated that joint pain is associated with an increased prevalence and severity of JE in joints affected by DDwR and DDwoR.[20] Joint effusion may be related to different inflammatory or non-inflammatory conditions in the TMJ, such as synovitis ($p = 0.031$) and adherences ($p = 0.042$), as highlighted by González et al.[41] In general, JE is statistically associated with various forms of internal derangement[18–23,31,42] and pain.

These findings are consistent with the correlation discovered between JE and pain in other joints, such as the shoulder, the knee and the ankle, as reported by various authors.[43–47]

Pain plays a crucial role in the diagnosis and treatment of patients. Its significance cannot be overstated, as it serves as the foundation upon which all medical examinations and treatments are based. It is important to keep this in mind when weighing the benefits of MRI against clinical examination in the diagnosis of TMJ-related pain. Magnetic resonance imaging is a method that requires careful evaluation in relation to the patient's clinical manifestation in order to avoid overinterpretation. Studies have revealed that even in cases of unilateral clinical symptoms, TMJs of both sides tend to exhibit similar combinations of MRI signs.[48,49] Asymptomatic JE does not require treatment, though this review suggests that it is much more frequently associated with pain than its absence.

The findings of the review indicate that pain experienced during clinical examination can serve as a reliable indicator of JE as observed through MRI. These outcomes bear significant clinical implications, since pain is often the primary reason for patients to seek medical attention and is frequently the foundation upon which clinicians base their therapeutic strategies. Therefore, recognizing the association between pain and JE can facilitate the process of differential diagnosis, leading to more effective outcomes. An extensive clinical assessment that takes into consideration 6 parameters, including pain, has been demonstrated to accurately predict the presence of JE on MRI in 78.7% of cases.[7] The clinical examination exhibits a high positive predictive value of 84.3%. In other words, the presence of clinically diagnosed pain is a reliable predictor of the presence of effusion in the TMJ.[7] Future studies should be directed toward the search for a more specific association with function-dependent symptoms, which may be influenced in a greater way by the presence of effusion than unspecific TMJ pain.

Future research directions

Despite the evidence presented in this systematic review, which supports a significant association between TMJ effusion and clinical pain, several limitations necessitate further research. One of the primary challenges is the heterogeneity of the included studies, particularly with regard to the methodologies used for pain assessment, imaging techniques and study populations. Future research should prioritize the standardization of these parameters to improve comparability and reproducibility of findings.

It is recommended that future studies employ uniform pain assessment tools that consider both subjective pain experiences and objective functional limitations. The use of standardized MRI protocols, including machine strength (1.5T vs. 3.0T) and imaging sequences, can ensure consistency in detecting effusion and other structural abnormalities.

The current body of literature is primarily composed of cross-sectional studies, which limit the ability to establish causal relationships between TMJ effusion and pain. Prospective, longitudinal studies that track patients over time can offer insight into the progression of JE and its impact on pain. Interventional studies assessing the correlation between the resolution of TMJ effusion and pain reduction could further validate its role as a clinical marker.

Given the evidence that psychological factors may influence TMJ pain perception, future research should incorporate psychological assessments to differentiate between pain of inflammatory origin and pain influenced by psychosocial factors. Biochemical markers of inflammation in TMJ effusion could provide additional objective measures to correlate with MRI findings and clinical symptoms**.**

A significant number of the reviewed studies exhibited relatively small sample sizes. The implementation of larger, multicenter trials could enhance statistical power and facilitate the acquisition of a more generalizable understanding of the relationship between effusion and pain across diverse populations.

Since TMJ effusion may be more strongly correlated with functional pain rather than with general TMJ discomfort, future research should specifically evaluate pain that is triggered by movement or function.

Advances in artificial intelligence (AI) could improve the detection and quantification of effusion on MRI scans. The application of AI-driven pattern recognition holds promise in the prediction of pain severity based on imaging findings.

By addressing these limitations, future studies can refine the clinical relevance of TMJ effusion as a diagnostic and prognostic marker. This approach is expected to enhance patient management by reducing the use of unnecessary imaging and improving targeted therapeutic interventions.

Conclusions

A substantial body of research has identified a significant association between pain and JE. This emphasizes the crucial role of pain in detecting this condition, and highlights the necessity for meticulous evaluation of patients with joint pain to minimize the reliance on costly, second-level diagnostic procedures, such as MRI. Moreover, the presence of TMJ pain in the absence of effusion may be considered quite atypical. Consequently, effusion should be prioritized as the primary factor to consider during pain evaluation to potentially explain function-dependent symptoms. In instances where clinical presentations deviate from the norm, characterized by the lack of association between pain and effusion, the diagnostic process should be directed toward identifying alternative sources of pain.

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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Review

Impact of human papillomavirus (HPV) infection on the development of oral squamous cell carcinoma (OSCC): A systematic review

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Abstract

This systematic review aimed to identify, select and synthesize clinical studies reporting the prevalence of HPV infection among patients with OSCC, and to determine the odds ratio (*OR*) of HPV infection in a group of OSCC patients relative to non-OSCC controls through meta-analysis.

The study incorporated primary clinical trials that assessed the impact of HPV infection on the development of OSCC. The search was conducted on August 31, 2023, using Bielefeld Academic Search Engine (BASE), as well as PubMed® and Scopus databases. The Newcastle–Ottawa Quality Assessment Scale was used to assess the risk of bias of the included studies. The collected data was then synthesized in the form of tables and a funnel plot. A total of 54 eligible studies were selected for the review, and 10 reports were included in the meta-analysis. Of the 10 papers, 7 reported extractable numerical data on HPV-16 and/or HPV-18 (1,035 patients).

The limitations of the evidence included the following: inhomogeneity in terms of HPV type; small number of available controlled studies (not homogeneous in terms of virus type); small number of patients on whom controlled studies were conducted; and the risk of bias related to the selection of study and control groups (present in most studies qualified for the synthesis).

In conclusion, HPV is detected by genetic testing in 0.0–74.5% of patients who develop OSCC. The weighted mean *OR* of detecting HPV-16 or HPV-18 in OSCC patients (*OR* = 17.1; standard deviation (*SD*) = 31.4) suggests a potential correlation between these infections and the incidence of OSCC.

Keywords: HPV, OSCC, oral squamous cell carcinoma, systematic review, human papillomavirus

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Highlights

- Human papillomavirus (HPV) infection is frequently detected in patients with oral squamous cell carcinoma (OSCC), with prevalence rates reaching up to 74.5% in genetic testing studies.
- Controlled studies show markedly higher odds of HPV-16 or HPV-18 detection in OSCC patients compared with non-OSCC controls (weighted mean $OR \approx 17$).
- Evidence linking OSCC with HPV types other than HPV-16 and HPV-18 remains limited and inconclusive.

Introduction

Background

Head and neck cancers account for over 5% of all malignancies.[1] This category includes cancers of the oral cavity, throat, larynx, paranasal sinuses, thyroid and salivary glands, as well as the surrounding soft and hard tissues.[2,3] Approximately 90% of cancers in this category are squamous cell carcinomas (SCCs).[3]

Oral squamous cell carcinoma (OSCC) is a term used to describe cancers with squamous cell differentiation developing within the oral mucosa and lips, excluding the skin of the mouth and the pharyngeal mucosa. Oropharyngeal SCC (OPSCC) refers to cancers located in the palatine tonsil, the root of the tongue, the glossotonsillar groove, and the mucous membrane of the lateral and posterior pharyngeal walls. In some publications,[4] the term OPSCC is used imprecisely to describe both cancer of the oral cavity and cancer of the oropharynx. However, current evidence supports the conclusion that OSCC and OPSCC are distinct and unique, with differing etiopathogenesis, treatment and prognosis.[3]

The risk of OSCC increases significantly after the age of 50, and the condition is diagnosed 3 times more often in males.[5] Despite advancements in technology, including self-learning systems, detecting oral cancer at an early stage remains challenging for clinicians.[6] Therefore, the identification of OSCC risk factors seems particularly important. The influence of tobacco smoking and chronic alcohol consumption on the development of OSCC has been extensively documented.[7] Additional significant risk factors include poor oral hygiene, chronic irritation of the mucous membrane due to faulty prosthetic restorations or dental fillings, candidiasis, and the presence of potentially malignant disorders such as leukoplakia, lichen planus or erythroplakia.[8,9]

Rationale

In recent years, there has been a sharp increase in the incidence of OSCC among patients in younger age groups (approx. 20–40 years) who developed OSCC despite good oral hygiene and the absence of any of the aforementioned risk factors.[5,10] Current research on the impact of head and neck cancer on the quality of life confirms that available treatment leads to its permanent deterioration (e.g., it induces sexual issues, which are of particular importance to this age group).[11] It has been speculated that in this group of patients, another initiator may be responsible for promoting the carcinogenesis process, and it is currently under debate whether high-risk human papillomavirus infection (HR-HPV) plays a role in the development of OSCC.[12] This hypothesis is related to the fact that, in relation to OPSCC, i.e., cancers developing in the immediate anatomical vicinity of the oral cavity, there is clear scientific evidence of the impact of HR-HPV infection on the development of cancer.[2] Recent studies have indicated that up to 70% of OPSCC cases are related to HR-HPV infection, primarily types 16 and 18.[13] However, the relationship between HPV infection and OSCC is still unclear.[14] The frequency of OSCC cases in which HR-HPV genetic material is detected, according to various authors, ranges between 2.6%[15] and 74%.[13] However, in most of the analyzed samples, HPV is detected in over 25% of OSCC cases.[16]

Revealing a direct relationship between viral infection and the occurrence of OSCC would have significant clinical implications. In the context of OPSCC, HPV(+) cancers are characterized by a less aggressive course and significantly better survival outcomes compared to HPV(–) cancers. Consequently, therapeutic interventions in this group of patients may be less invasive.[17,18]

Aim

The aim of this systematic review was to identify, select and synthesize clinical studies reporting the prevalence of HPV infection among patients with OSCC, and to determine the odds ratio (*OR*) of HPV infection in a group of OSCC patients relative to non-OSCC controls through meta-analysis. The collection of this data is intended to indirectly assess whether the risk of OSCC is greater in HPV-positive individuals.

Material and methods

This systematic review is based on and arranged in accordance with the current version of the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[19] The completed PRISMA checklist and the PRISMA checklist for abstracts constitute the supplementary material to this article (available on request from the corresponding author). This systematic review has been registered in the PROSPERO database (registration No. CRD42023483769).

Eligibility criteria

Primary clinical trials published in English that evaluated the impact of HPV infection on the development of OSCC were included. The detailed eligibility criteria are presented in Table 1, albeit the Control and Outcomes criteria only concerned the inclusion in the meta-analysis.

Information sources

A comprehensive search of medical databases was conducted using the Bielefeld Academic Search Engine (BASE), PubMed® and Scopus. All final searches were performed on August 31, 2023.

Search strategy

The search strategy, identical for each of the engines, was formulated based on the eligibility criteria and arranged in the form of the following query: ("oscc" OR "oral scc" OR "oral squamous cell carcinoma") AND ("hpv" OR "papillomavirus" OR "papilloma virus") AND ("correlation" OR "correlated" OR "effect" OR "affects" OR "impact" OR "influence" OR "link" OR "connection") AND ("study" OR "trial" OR "primary").

Article selection

The identified records were entered into the Rayyan automation tool (Rayyan Systems, Cambridge, USA).[20]

Table 1. Eligibility criteria for study inclusion and exclusion

PICOS	Criteria for inclusion	Criteria for exclusion
Population, Problem	OSCC diagnosis	animal studies
Intervention	detection of the HPV	methods not detecting the genome
Control*	non-OSCC group	none
Outcomes*	*OR*	none
Timeframe	unlimited	preprints
Setting	primary studies on groups of 10 and more	none

* applied to the meta-analysis only; OSCC – oral squamous cell carcinoma; HPV – human papillomavirus; *OR* – odds ratio.

The duplicates were automatically removed, and the records indicated by the tool as potential duplicates were manually verified (IR and MC). A blind screening of abstracts and titles was performed by 2 researchers (IR and MC). The convergence of researchers' ratings was expressed by Cohen's kappa coefficient. In cases of non-compliance during the screening stage, the record underwent further processing. The full-text reports were evaluated by the same researchers (IR and MC). Discrepancies that emerged during the full-text evaluation phase were resolved through consensus.

Data collection

The data was extracted from the source articles by 2 independent authors (IR and MC). The present study exclusively utilized published data, specifically the content of articles and supplementary materials. The collection process did not involve the use of automation tools.

Extracted characteristics of the studies

The following items were extracted from the source studies: first author and year of publication; sex and average age of patients; OSCC location; total number of patients in the OSCC group; number of patients in the OSCC group with genetically confirmed HPV; total number of patients in the non-OSCC group; number of patients in the non-OSCC group with genetically confirmed HPV; *OR* between HPV and OSCC.

Assessment of the risk of bias

The Newcastle–Ottawa Quality Assessment Scale was used to assess the risk of bias for studies included in the meta-analysis. The evaluation was performed by 2 independent researchers (IR and MC). Any discrepancies in assessment were resolved through consensus, and no automation tools were implemented in the process.

Effect measures

For studies with a control group, the *OR* was calculated using the MedCalc Statistical Software v. 22.018 (MedCalc Software Ltd, Ostend, Belgium).

Data synthesis

The collected data was synthesized in the form of tables and a funnel plot using Google Workspace (Google LLC, Mountain View, USA).

Reporting the assessment of bias

In instances of missing data, this fact was noted, yet the series was not discarded. No further reporting bias assessments were undertaken.

Results

Study selection

The number of records identified from each search engine/database is presented in Table 2. During the selection process, 54 eligible studies were selected out of 1,325 identified records (Fig. 1). The consistency index of the judges' assessments at the screening stage was $\kappa = 0.87$, which represents almost perfect agreement. The study by Cao et al. was considered eligible based on the abstract, but its full text was not obtained due to the lack of digital archiving of articles from these years in the Chinese Journal of Dental Research.[21]

Study characteristics

Fifty-four studies included in the review are summarized in Table 3. Each study considered different locations of OSCC, making it impossible to create subgroups based on specific areas of the oral cavity. The collected material covered clinical trials since 1996, which resulted in an overview of over a quarter of a century of active research on the relationship between HPV infection and the occurrence of OSCC. The study samples did not exceed 254 patients, and most reports were based on material from fewer than 100 subjects. The preponderance of single-center studies made it challenging to extrapolate results to broader populations. The percentage of diagnosed HPV infections in OSCC patient groups fluctuated significantly (0.0–74.5%), which was partly related to the limited sample size and the heterogeneity of the identified virus types. The majority of the papers did not include a control sample and were limited to a single arm, precluding the possibility of synthesizing them to ensure a high level of evidence for the review. Therefore, studies incorporating a control group advanced to further stages of the review.

Table 2. Number of records identified through database search

Search engine/database	Coverage, *n*	Identified records, *n*
BASE	340,488,332	629
PubMed®	>36,000,000	365
Scopus	>87,000,000	331
Total		1,325

BASE – Bielefeld Academic Search Engine.

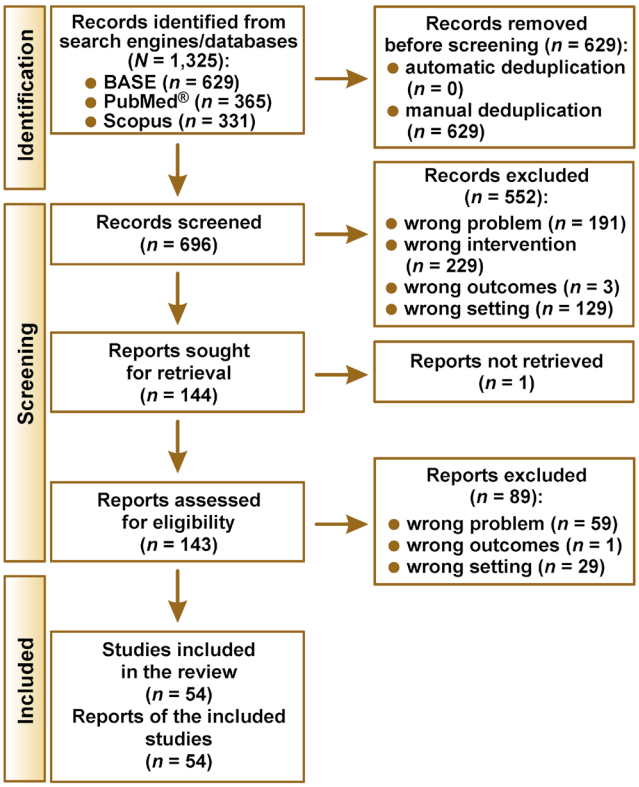

Fig. 1. Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) flowchart of the study

BASE – Bielefeld Academic Search Engine.

Risk of bias

The Newcastle–Ottawa Quality Assessment Scale scores for each of the studies included in the meta-analysis ranged from 6 to 9 (maximum) points (Table 4). Therefore, the overall risk of bias for the studies was low or raised some concerns. The ratings were decreased primarily due to the absence of statements regarding the inclusion of consecutively reporting patients and limited information on the control groups.

Results of individual studies

The results of individual studies are outlined in Table 5. Most studies had symmetrical sample sizes, though there were exceptions to this rule. The number of patients in the study groups ranged from 30 to 200, which enabled quantitative analysis. Depending on the study, the sex ratio was either similar or men predominated by up to 2.5 times. The percentage of HPV infections in the control samples did not exceed 55%. In numerous reports, the prevalence of HPV infection in the control sample was negligible or equal to 0. In each study sample, HPV was detected in at least 1 patient, and the percentage of infected individuals reached up to 74%. Interestingly, a study that encompassed a broader range of virus types observed a lower infection rate in comparison to the maximum recorded value.[42] This could be due to the lack of identification of HPV-18, which is present in the majority of tests conducted by other research groups. Discrepancies can be observed in relation to the type of identified virus, with a clear dominance of HPV-16 and HPV-18. In studies with smaller sample sizes, the *OR* did not attain statistical significance. In cases with confirmed statistical significance, the *OR* ranged from 2.3 to 86.3, indicating a higher probability of identifying HPV in materials from patients diagnosed with OSCC.

Table 3. Characteristics of studies included in the review

Study	Country	Presence of a control group (*n*)	OSCC group, *n*	Males/females in the OSCC group (control group), *n*	Mean age in the OSCC group (control group) [years]	HPV infections in the OSCC group [%]	HPV type
Mneimneh et al. 2021[10]	USA	no	150	89/61	34.0	0.0	16
Saleh et al. 2023[22]	USA	no	114	56/58	70.8	12.0	16
Al-Dabbagh et al. 2022[23]	UK	no	124	85/39	60.0	0.0	16
Yang et al. 2019[24]	China	yes (30)	30	14/16 (14/16)	58.0 (50.0)	3.3	67, 68
Loeschke et al. 2016[25]	Germany	no	91	59/32	55.7	7.7	16
Popović et al. 2010[26]	Serbia	no	60	47/13	N/S	10.0	16
Zhao et al. 2009[17]	China	no	52	35/17	N/S	40.4	6, 16, 18
Oliveira et al. 2008[27]	Brazil	no	87	73/14	N/S	19.5	16, 18
de Freitas Cordeiro-Silva et al. 2012[5]	Brazil	no	45	35/10	58.0	6.0	16
Singh et al. 2015[12]	India	no	250	200/50	N/S	9.2	16, 18
Adnan Ali et al. 2018[28]	Pakistan	no	140	82/58	N/S	67.9	16, 18
Gan et al. 2014[29]	China	yes (68)	200	143/57 (27/41)	N/S (N/S)	27.5	16, 18
Rivero and Nunes 2006[30]	Brazil	no	40	32/8	57.0	0.0	16, 18
Sivakumar et al. 2021[31]	India	no	26	23/3	58.2	14.0	16
Duray et al. 2012[32]	Belgium	no	147	N/S	N/S	70.1	16, 45, 53, 58, 59, 66, 67
Grewal et al. 2018[33]	India	no	47	36/11	46.7	74.5	16, 18
Schwartz et al. 2001[18]	USA	no	254	162/92	54.2	15.1	16
Rushatamukayanunt et al. 2014[34]	Japan	yes (40)	40	N/S (N/S)	31.5 (61.0)	5.0	16, 18
Chen et al. 2016[7]	China	yes (189)	178	110/68 (117/72)	58.9 (56.6)	14.0	16, 18
Polz-Gruszka et al. 2015[35]	Poland	no	154	131/23	56.8	30.4	6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68, 69, 70, 71, 73, 74, 82
Jalouli et al. 2010[36]	India	no	62	50/12	58.2	24.0	16, 18
Chen et al. 2012[37]	Taiwan	no	65	52/13	53.0	36.9	16
Zhang et al. 2004[13]	China	yes (40)	73	48/25 (24/16)	N/S (N/S)	74.0	16, 18
Premoli-De-Percoco and Ramirez 2001[38]	Venezuela	no	50	0/50	48.2	60.0	6, 11, 16, 18
Antunović et al. 2022[39]	Montenegro	no	60	47/13	62.0	23.3	16
Chen et al. 2016[40]	China	no	40	29/11	N/S	0.0	16, 18
Campisi et al. 2006[41]	Italy	no	63	28/35	68.9	38.1	16, 18
Saini et al. 2011[42]	Malaysia	yes (105)	105	51/54 (58/47)	56.2 (42.0)	51.4	6, 11, 16, 26, 31, 33, 35, 45, 51, 53, 54, 58

Study	Country	Presence of a control group (*n*)	OSCC group, *n*	Males/females in the OSCC group (control group), *n*	Mean age in the OSCC group (control group) [years]	HPV infections in the OSCC group [%]	HPV type
Termine et al. 2012[43]	Italy	no	83	43/40	64.0	15.7	16, 39, 51
Nagpal et al. 2002[44]	India	no	110	68/42	N/S	33.6	16, 18
Laco et al. 2012[1]	Czech Republic	no	48	N/S	N/S	15.0	16
Lee et al. 2012[14]	Taiwan	no	173	N/S	N/S	22.0	16, 18
Penhallow et al. 1998[9]	UK	no	28	N/S	65.6	50.0	6,16
Nemes et al. 2006[45]	Hungary	no	79	67/12	55.8	41.8	16
Correnti et al. 2004[46]	Venezuela	no	16	7/9	54.0	50.0	16, 18
Premoli-De-Percoco et al. 1998[47]	Venezuela	no	50	0/50	55.0	70.0	6, 11, 16, 18
González-Moles et al. 1996[48]	Spain	no	37	26/11	60.0	19.1	18
Cortés-Gutiérrez et al. 2021[15]	Mexico	yes (10)	38	N/S (N/S)	43.0 (45.5)	2.6	16
Shima et al. 2000[49]	Japan	no	46	32/14	N/S	73.9	16, 18
Ali et al. 2008[50]	Pakistan	no	140	N/S	N/S	68.0	16, 18
Tyagi et al. 2019[51]	Nepal	yes (50)	50	29/21 (N/S)	48.6 (N/S)	46.0	16, 18
Sharma and Prakash 2023[52]	India	no	15	7/8	50.8	26.7	N/S
Soares et al. 2003[53]	Brazil	no	27	N/S	N/S	40.7	6, 11, 16, 18
Tabatabai et al. 2015[54]	Iran	yes (27)	39	22/17 (18/9)	64.2 (63.6)	43.6	16, 18
Pandey et al. 2018[55]	India	no	24	21/3	53.1	20.8	16, 18
de Lima et al. 2022[56]	Brazil	no	100	64/36	N/S	31.0	6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52
Ibieta et al. 2005[57]	Mexico	no	51	37/14	N/S	42.0	16, 18
Ali et al. 2014[58]	Pakistan	no	140	N/S	60.3	68.0	16, 18
Yang et al. 2004[59]	Taiwan	yes (36)	37	N/S (N/S)	N/S (N/S)	10.8	16, 18
Panzarella et al. 2021[60]	Italy	no	40	23/17	66.5	10.0	16, 31, 51, 66, 67, 68
Ono et al. 2014[61]	Japan	no	93	59/34	N/S	10.7	6, 11, 16, 18, 22, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 83, 84
Ali et al. 2014[62]	Pakistan	no	140	N/S	N/S	68.0	16, 18
Kozomara et al. 2005[63]	Montenegro	no	50	42/8	55.4	64.0	16, 18, 31, 33
Ali and Awan 2016[64]	Pakistan	no	140	N/S	N/S	67.9	16, 18

N/S – not specified.

Table 4. Assessment of the risk of bias for studies included in the meta-analysis

Study	Newcastle–Ottawa Quality Assessment Scale			
	Selection	Comparability	Exposure	Sum (0–9)
Yang et al. 2019[24]	1-1-1-1	2	1-1-1	9
Gan et al. 2014[29]	1-0-1-1	2	1-1-1	8
Rushatamukayanut et al. 2014[34]	1-0-0-0	2	1-1-1	6
Chen et al. 2016[7]	1-0-0-1	2	1-0-1	6
Zhang et al. 2004[13]	1-0-1-0	2	1-1-1	7
Saini et al. 2011[42]	1-0-1-1	2	1-1-1	8
Cortés-Gutiérrez et al. 2021[15]	1-0-1-1	2	1-1-1	8
Tyagi et al. 2019[51]	1-0-1-1	2	1-1-1	8
Tabatabai et al. 2015[54]	1-0-0-0	2	1-1-1	6
Yang et al. 2004[59]	1-0-0-1	2	1-1-1	7

Results of data synthesis

Of the 10 reports containing numerical data that enabled *OR* calculation, 8 assessed HPV-16 and/or HPV-18; however, only 7 studies provided extractable data specific to HPV-16 or HPV-18. The *OR* results for HPV-16 and HPV-18 were synthesized in a funnel plot (Fig. 2). This synthesis was based on a total of 1,035 patients. The weighted mean *OR* was 17.1 (standard deviation (*SD*) = 31.4). The *OR* result reported in the study by Tyagi et al. was an outlier.

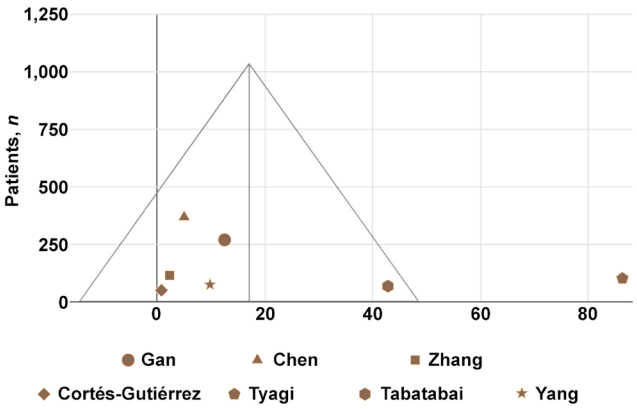

Fig. 2. Funnel plot displaying odds ratios (x-axis) derived from reports on human papillomavirus (HPV)-16 and/or HPV-18

Discussion

General interpretation of the results

In the collected research material, the percentage of OSCC patients infected with HPV ranged from 0.0% to 74.5%. Significant discrepancies in the results have been confirmed in other reviews.[65,66] The discrepancies are likely attributable to the testing of different types of the virus within individual clinical trialws. Moreover, the method of testing for the HPV genome, even of the same type, is not uniform, which may be the reason for differences in the results presented by different teams of researchers.

Table 5. Results of individual studies included in the meta-analysis

Study	Non-OSCC group (male/female), *n*; mean age [years]	HPV infections in the non-OSCC group, *n* (%)	OSCC group (male/female), *n*; mean age [years]	HPV infections in the OSCC group, *n* (%)	*OR* and significance level	HPV type
Yang et al. 2019[24]	30 (14/16) 50.0	1 (3.3)	30 (14/16) 58.0	1 (3.3)	1.0 $p = 1.00$	67, 68
Gan et al. 2014[29]	68 (27/41) N/S	2 (2.9)	200 (143/57) N/S	55 (27.5)	12.5 $p < 0.05$	16, 18
Rushatamukayanut et al. 2014[34]	40 (N/S) 61.0	1 (2.5)	40 (N/S) 31.5	2 (5.0)	2.1 $p = 0.56$	N/S
Chen et al. 2016[7]	189 (117/72) 56.6	6 (3.2)	178 (110/68) 58.9	25 (14.0)	5.0 $p < 0.05$	16, 18
Zhang et al. 2004[13]	40 (24/16) N/S	22 (55.0)	73 (48/25) N/S	54 (74.0)	2.3 $p < 0.05$	16, 18
Saini et al. 2011[42]	105 (58/47) 42.0	26 (24.8)	105 (51/54) 56.2	54 (51.4)	3.2 $p < 0.05$	6, 11, 16, 26, 31, 33, 35, 45, 51, 53, 54, 58
Cortés-Gutiérrez et al. 2021[15]	10 (N/S) 45.5	0 (0.0)	38 (N/S) 43.0	1 (2.6)	0.8 $p = 0.92$	16
Tyagi et al. 2019[51]	50 (N/S) N/S	0 (0.0)	50 (29/21) 48.6	23 (46.0)	86.3 $p < 0.05$	16, 18
Tabatabai et al. 2015[54]	27 (18/9) 63.6	0 (0.0)	39 (22/17) 64.2	17 (43.6)	42.8 $p < 0.05$	16, 18
Yang et al. 2004[59]	36 (N/S) N/S	0 (0.0)	37 (N/S) N/S	4 (10.8)	9.8 $p = 0.13$	16, 18

Of the 54 eligible studies, only 10 included a control group. The ideal study design would involve a study group and a control group that are matched in terms of sex and age. In the available material, control groups were selected primarily from patients of the same institution (but not from the general population).

The results of the *OR* for the presence of the HPV genome in OSCC vs. non-OSCC patients indicate the presence of a relationship between the occurrence of OSCC and HPV-16 or HPV-18 infection. With the exception of the study by Cortés-Gutiérrez et al.,[15] which was conducted on a small group of patients, the *OR* for the HPV-16 and HPV-18 in OSCC vs. non-OSCC groups is greater than 1, indicating a higher probability of detecting these types of virus in patients with OSCC. Based on the patient-derived outcomes, it was confirmed that a positive result for HPV-16 and/or HPV-18 is associated with a significant risk of developing OSCC.

The collected research material is insufficient to draw similar conclusions regarding other types of the virus. The study by Yang et al. showed no statistically significant relationship between the incidence of OSCC and the presence of HPV-67 or HPV-68 infection.[24]

Biological risk factors for OSCC

The current state of knowledge suggests a correlation between HPV and the risk of OSCC, which is the subject of this paper. Additionally, an increased predisposition to OSCC is suspected in patients infected with Epstein–Barr virus (EBV).[56] A similar relationship has been observed among individuals infected with bacteria that cause periodontitis, i.e., *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. However, there is a lack of clinical evidence for this relationship, and assumptions are solely based on in vitro and animal studies.[67] The previously ambiguous relationship between *Candida* spp. infection and the development of OSCC was confirmed in a systematic review from 2023.[68] The demonstrated correlation supports the possibility of causality, which requires further research.

HPV diagnostics

The gold standard for the diagnosis of HPV infection is the identification of the genetic material of the virus by polymerase chain reaction (PCR). An alternative method involves the identification of cyclin-dependent kinase inhibitor 2A (p16) protein immunohistochemically.[16] A meta-analysis of diagnostic methods for oral HPV showed high sensitivity but moderate specificity of immunohistochemical identification.[69] It is, therefore, accepted that the detection of p16 is of high value as a screening test, but may not be sufficient for scientific purposes. The present systematic review was based solely on source studies, in which the presence of HPV genetic material in patient tissues was confirmed by PCR.

Benign lesions

The infection with HPV may also contribute to the development of benign lesions, commonly referred to as oral warts. These include, among others, focal epithelial hyperplasia (Heck's disease), squamous cell papilloma of the oral cavity, common wart (verruca vulgaris), and condyloma acuminata of the oral cavity.[70] For a progression to a malignant state, the presence of appropriate co-factors is necessary, including genetic predisposition, smoking and alcohol consumption.[71]

The significance of HPV infection in oral potentially malignant lesions, specifically in leukoplakia, remains unclear.[72] A recent systematic review has estimated the overall HPV prevalence in leukoplakia at 6.66%, whereas the prevalence of HPV-16 at 2.95%.[73] However, the current update from the World Health Organization (WHO) classification of head and neck tumors distinguishes the HPV-associated dysplasia as a separate lesion, with about 15% risk of malignant transformation.[74,75]

Future research

A particular emphasis must be placed on the methodological problem that is present in all qualified studies. The authors of the source papers attempted to estimate the risk of OSCC among HPV(+) vs. HPV(–) patients by designing studies in which the presence of the viral genome was determined in OSCC and non-OSCC patients. In order to most appropriately investigate the incidence of OSCC among patients with HPV, a long-term follow-up of HPV(+) vs. HPV(–) cohorts in the context of OSCC occurrence is necessary. It is important to consider the possibility of HPV infection during observation, and to differentiate between the various types of the virus.

Limitations of the evidence

The limitations of the evidence included the following: inhomogeneity in terms of HPV type; small number of available controlled studies (not homogeneous in terms of virus type); small number of patients on whom controlled studies were conducted; and the risk of bias related to the selection of study and control groups (present in most studies qualified for the synthesis).

Limitations of the review process

The main limitation of the review process included the use of a query in English, thereby excluding reports that lacked at least a title or abstract in this language. Moreover, the search engine and databases used do not ensure the identification of articles published in locally indexed journals.

Conclusions

Human papillomavirus is detected through genetic testing in 0.0–74.5% of patients with OSCC. The weighted average *OR* for detecting HPV-16 or HPV-18 in OSCC patients is 17.1, suggesting that these viral variants may contribute to the development of OSCC.

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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Review

Checkpoint inhibitors in squamous cell carcinoma of the head and neck: History and new perspectives

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Abstract

This review aims to comprehensively examine the historical development, molecular mechanisms and clinical applications of checkpoint inhibitors in squamous cell carcinoma of the head and neck (SCCHN). Squamous cell carcinoma of the head and neck represents a significant global health challenge as the 7th most common malignancy worldwide. Immune checkpoint inhibitors (ICIs) targeting the PD-1/PD-L1 and CTLA-4 pathways have emerged as promising therapeutic approaches. Current evidence supports the use of ICIs in the recurrent/metastatic (R/M) setting, while data for neoadjuvant and adjuvant applications is evolving. Pembrolizumab monotherapy or in combination with chemotherapy has demonstrated survival benefits in PD-L1-positive R/M SCCHN, while nivolumab has shown efficacy in the second-line setting. Results from trials combining ICIs with radiotherapy have been mixed, with several phase III studies failing to meet primary endpoints.

The integration of ICIs has transformed the treatment landscape for R/M SCCHN, while the ongoing research continues to define their optimal use in earlier disease settings and in novel therapeutic combinations. Future directions include exploring combination strategies with targeted therapies, identifying predictive biomarkers beyond PD-L1 expression, and developing immunotherapy approaches tailored to HPV-positive vs. HPV-negative disease.

Keywords: CTLA-4, PD-1, PD-L1, head and neck squamous cell carcinoma, checkpoint inhibitors

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Highlights

- Squamous cell carcinoma of the head and neck (SCCHN) represents a significant global health challenge.
- Immune checkpoint inhibitors (ICIs) are one of most promising paths in the therapy of SCCHN.
- Immunotherapy could be used in different phases of treatment as neoadjuvant therapy, adjuvant therapy after definitive surgery, in combination with definitive (radical) radiotherapy, or as palliative care.
- Pembrolizumab and nivolumab have been integrated into routine clinical practice for metastatic and relapsed SCCHN.

Introduction

Head and neck cancer has emerged as a global health challenge, representing the 7th most common malignancy worldwide, with an annual incidence of 890,000 cases and around 450,000 deaths in 2022.[1] Squamous cell carcinomas constitute approx. 90% of all head and neck cancers (SCCHN).[2] The prevalence of SCCHN is significantly higher in developing countries.[3] Environmental carcinogens, particularly tobacco and alcohol, are responsible for 75–85% of SCCHN cases.[4] The molecular pathogenesis of SCCHN frequently involves mutations in the tumor protein p53 gene (*TP53*), induced by xenobiotics that interfere with DNA synthesis and repair mechanisms. *TP53* mutations are associated with shorter overall survival (OS), potential therapeutic resistance and increased recurrence rates.[5] Human papillomavirus (HPV) infection represents a second major etiological factor, particularly in oropharyngeal cancers. HPV-positive patients constitute approx. 30–35% of oropharyngeal cancer cases and 6% of all oropharynx cancers. In contrast to *TP53*-mutated tumors, HPV-positive disease is associated with significantly better outcomes.[6,7] Furthermore, the role of Epstein–Barr virus (EBV) infection in the development and progression of tumor cells has been proven in head and neck cancers, such as nasopharyngeal cancer.[8] In case of cytomegalovirus (CMV), oncogenic potential in head and neck cancer is unclear.[9] Additional risk factors include radiation exposure, poor oral hygiene, inadequate nutrition, betel nut chewing, ill-fitting dentures, and certain genetic syndromes, such as Fanconi anemia, ataxia–telangiectasia, Bloom's syndrome, Li–Fraumeni syndrome, and dyskeratosis congenita.[8] The association between periodontal disease,[9] gut microbiota[10] and cancer risk has also been postulated.

The molecular mechanisms of head and neck carcinogenesis comprise genetic and epigenetic alterations, which lead to the malignant neoplastic process. Among genetic factors, the most important are mutated oncogenes *p53* or *RAS*, as well as the inactivation of tumor suppressor proteins like p16INK4a.[11] Uncontrolled proliferation and apoptosis evasion – typical for cancer – are connected with such pathways as the epidermal growth factor receptor (EGFR), phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR), Janus kinase/signal transducer and activator of transcription (JAK/STAT), and Wnt/β-catenin.[12] DNA methylation, histone acetylation and methylation, as well as microRNA-mediated regulation, are among the most probable epigenetic factors involved in the tumorigenesis of head and neck cancers.[13]

While advances in diagnostic techniques, including artificial intelligence (AI)-based approaches and neural networks, have improved early detection,[14] novel therapeutic strategies are needed for advanced-stage disease to complement or replace conventional chemotherapy and radiotherapy. Immune checkpoint inhibitors (ICIs) have emerged as one of the most promising approaches in the systemic treatment of SCCHN. In patients who may potentially benefit from ICI therapy, programmed death ligand-1 (PD-L1) expression is evaluated using the combined positive score (CPS), defined as the number of PD-L1-positive cells (tumor cells, lymphocytes and macrophages) divided by the total number of tumor cells, multiplied by 100.[15,16]

Despite the growing amount of literature on immunotherapy in SCCHN, most of the existing reviews focus either on the general mechanisms of immune checkpoint inhibition or on specific clinical trials, without integrating historical context, biomarker-driven strategies and the emerging therapeutic combinations. There is a lack of comprehensive narrative reviews that would bridge the evolution of checkpoint inhibitors with current clinical applications and future directions in SCCHN treatment.

This review addresses that gap by aiming to provide a comprehensive overview of the historical development and current vision of checkpoint inhibitor therapy in SCCHN, highlighting key clinical trials, the emerging therapeutic targets and future directions in immunotherapy.

Material and methods

The literature search was performed using the PubMed, Scopus and Web of Science electronic databases.

We included peer-reviewed articles published between 1982 and May 2024, written in English, that addressed the use of checkpoint inhibitors in the treatment of SCCHN. Both clinical trials and high-quality narrative or systematic reviews were considered.

Studies were included if they met the following criteria: studies involving adult patients with SCCHN; articles discussing checkpoint inhibitors as monotherapy or in combination; reviews and clinical trials with clearly reported outcomes. Exclusion criteria were as follows: non-English publications; case reports; editorials; studies focusing on non-squamous histology or unrelated cancer types.

Immune checkpoint inhibitors (ICIs) – biological basis and development

The field of cancer immunotherapy was revolutionized by the pioneering work of Tasuku Honjo from Kyoto University, Japan, and James Patrick Allison from MD Anderson Cancer Center, Houston, Texas, who were awarded the Nobel Prize in Physiology and Medicine in 2018 for their discovery of cancer therapy through the inhibition of negative immune regulation.[17]

PD-1/PD-L1 pathway

Honjo's research group first isolated the complementary DNA (cDNA) of programmed death receptor-1 (PD-1)[18] and demonstrated its role as a negative regulator of B cell responses, particularly in antibody class switching.[19] PD-1 is a member of the immunoglobulin superfamily and the cluster of differentiation (CD)28/cytotoxic T-lymphocyte associated protein-4 (CTLA-4) subfamily, expressed on CD8+ and CD4+ T cells, natural killer (NK) cells, B cells, and tumor-infiltrating lymphocytes (TILs).[20] PD-1 expression is induced by cytokines interleukin (IL)-2, IL-7, IL-15, and IL-21.[21] Additionally, ICIs can influence the cytokine environment; for example, avelumab reduces STAT3 expression, affecting interleukin -17 receptor A (IL-17RA) and CD15.[22]

The characteristic feature of the immunoglobulin (Ig) superfamily is a single Ig V-like domain in the extracellular region, which is crucial for binding to ligands.[23] The PD-1 structure comprises 3 parts: a ~20-amino acid stalk; a transmembrane domain; and a cytoplasmic tail with 2 tyrosine-based signaling motifs. The N-terminal extremity sequence contains an immunoreceptor tyrosine-based inhibitory motif (ITIM), called VDYGEL, which recruits SH2 domain-containing phosphatases.[24] The elements responsible for the inhibitory function of PD-1, the sequence TEYATI and immunoreceptor tyrosine-based switch motif (ITSM), are located at the C-terminal extremity.[25]

The ligation of PD-1 leads to the formation of PD-1/T-cell receptor (TCR) inhibitory micro-clusters, and recruits SHP1/2. Simultaneously, ITIM and ITSM sequences are dephosphorylated by Src-family tyrosine kinases. The intracellular pathways Ras GTPase/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (RAS/MEK/ERK) and PI3K/AKT are activated through the recruitment of SHP1 and SHP2. SHPs can also block protein kinase C theta (PKC-θ) and ZAP-70.[26] Consequently, this sequence arrests the cell cycle and suppresses T cell activation through the induction of apoptosis, the reduction of proliferation and the inhibition of cytokine secretion.[27,28]

Programmed death ligand-1 (PD-L1), also known as protein B7-H1 or CD274, was identified through the collaboration of the Honjo and Freeman research groups.[29] This ligand is encoded on chromosome 9p24.1 in the *CD274* gene,[30] and is expressed on the surface of T cells, B cells, macrophages, dendritic cells, mesenchymal stem cells, and bone marrow-derived mast cells.[31] PD-L1 binding can increase T-cell proliferation, decrease IL-2 secretion and increase IL-10 secretion.[32] PD-L2 (CD273 or B7-DC) is encoded by the *PDCD1LG2* gene on chromosome 9p24.1.[32] This protein was isolated by Latchman and colleagues,[33] and is expressed on activated CD4+ or CD8+ cells, dendritic cells, macrophages, and bone marrow-derived mast cells.[34,35] The interaction between PD-L1 located on tumor cells and PD-1 on T cells can diminish the immunological response to neoplastic disease by suppressing T cell activation. Multiple cytokines are identified as biomarkers for the diagnosis, prognosis and treatment of oral squamous cell carcinoma.[36]

The rapid development of PD-1 inhibitors (nivolumab, pembrolizumab, dostarlimab, cemiplimab) and PD-L1 inhibitors (atezolizumab, avelumab, durvalumab) has revolutionized the systemic treatment of various cancers.[36]

CTLA-4 pathway

In 1991, James Patrick Allison, the second "father of immunotherapy," discovered CTLA-4 and demonstrated its inhibitory role in anti-tumor T-cell activity.[37,38] In 1995, CTLA-4 was identified as a negative regulator of T-cell activation.[39] The receptor, also known as CD152, is a member of the Ig superfamily responsible for recruiting phosphatases to TCRs and attenuating their signals.[40] CTLA-4 is also found in regulatory T cells and dendritic cells.[41] In 1996, Allison demonstrated that the blockade of CTLA-4 could enhance anti-tumor immune responses, opening a second avenue for ICI therapy.[42] In 2011, the Food and Drug Administration (FDA) approved the first anti-CTLA-4 antibody, ipilimumab, for the treatment of metastatic melanoma.[43] Subsequently, CTLA-4 blockade has become an integral component of therapeutic regimens for SCCHN.[44] The emerging data suggests that another antibody from this group, tremelimumab, is being investigated for application in SCCHN therapy.[45]

Neoadjuvant therapy before definitive surgery

The anatomical location of head and neck cancers often necessitates disfiguring surgical or radiotherapeutic procedures, which can significantly impair quality of life after radical therapy. The implementation of neoadjuvant or concurrent (with radiotherapy) systemic treatment using ICIs could potentially reduce complications and improve cosmetic outcomes, thereby enhancing quality of life and prolonging disease-free survival (DFS). Furthermore, tumor downstaging may render previously unresectable lesions amenable to surgical resection and reduce the risk of positive surgical margins. Upfront surgery and neoadjuvant chemotherapy were compared in a retrospective study.[46]

Recent years have witnessed numerous investigations focusing on neoadjuvant ICI therapy. One of the earliest studies was the phase II trial NCT02296684, in which 14 patients received 2 doses of neoadjuvant pembrolizumab before surgical intervention for head and neck cancer.[47] A substantial pathological tumor response (pTR) (≥50%) was observed in 45% of participants. Single-cell analysis of 17,158 CD8+ T cells revealed that the responding tumors had clonally expanded putative tumor-specific exhausted CD8+ TILs with a tissue-resident memory program, characterized by high cytotoxic potential (CTX+) and ZNF683 expression. Five weeks after therapy, the effect was consistent with the activation of the pre-existing CTX+ZNF683+CD8+ TILs and associated with high numbers of CD103+PD-1+CD8+ T cells infiltrating pre-treatment lesions. In non-responders, the absence of ZNF683+CTX+ TILs correlated with the subsequent accumulation of highly exhausted clones. These observations suggest the important role of the pre-existing ZNF683+CTX+ TILs in the primary mechanism of response following neoadjuvant treatment.[47]

Another PD-1 inhibitor, nivolumab, was evaluated in patients with resectable HPV-positive and HPV-negative SCCHN in the phase I/II clinical trial CheckMate 358.[48] This study included 26 HPV-positive and 26 HPV-negative participants who received nivolumab 240 mg intravenously on days 1 and 15, with surgery scheduled by day 29. Radiographic responses were achieved in only 12.0% of HPV-positive and 8.3% of HPV-negative patients, with pathological responses in 5.9% and 17.6% of participants, respectively. A partial pathological response (pPR) was confirmed in only one HPV-positive patient, with no complete pathological responses (pCR) observed. Despite these modest response rates, treatment-related adverse events of any grade occurred in 73.1% of HPV-positive patients and 53.8% of HPV-negative patients, with grade 3–4 events in 19.2% and 11.5%, respectively.[48]

Several trials also investigated the combination of ICIs with chemotherapy. For example, a phase II clinical trial evaluated a single dose of durvalumab with or without tremelimumab before resection.[49] The study enrolled 48 patients, randomized into 2 arms: 24 patients received the combination therapy; and 24 received durvalumab monotherapy. From the entire cohort, 45 underwent surgical resection followed by postoperative chemoradiotherapy or radiotherapy based on multidisciplinary assessment, with 1-year consolidation with durvalumab. Distant recurrence-free survival (DRFS) was significantly better in patients treated with combination therapy as compared to the monotherapy arm. Artificial intelligence-powered analysis demonstrated that combination therapy reshaped the tumor microenvironment toward immune-inflamed phenotypes, in contrast to monotherapy or cytotoxic chemotherapy. The authors concluded that a single dose of durvalumab with tremelimumab before resection followed by postoperative chemoradiotherapy could benefit patients with resectable head and neck cancers.[49]

In another phase II randomized trial, neoadjuvant nivolumab monotherapy was compared to ICI doublet therapy – ipilimumab plus nivolumab or relatlimab plus nivolumab – for 4 weeks prior to surgery.[50] Participants were stratified by p16, PD-L1 and lymphocyte-activation gene 3 (LAG-3) expression, assessed with immunohistochemistry. Of the 41 patients enrolled, only 33 were evaluable for analysis (25 with oral cavity cancer, 5 with oropharyngeal cancer, and 3 with laryngeal cancer). In the doublet arms, pathological responses were more frequent (nivolumab/relatlimab: 11/13 and nivolumab/ipilimumab: 6/10) than in the nivolumab monotherapy arm (6/10). The combination arms were also associated with more partial (>50%) or major (>90%) pathological responses than monotherapy. There was no association between the RECIST (Response Evaluation Criteria in Solid Tumors) response, PD-L1 or LAG3 expression and the pathological response in the nivolumab/relatlimab arm; however, more patients with combined positivity had a >50% response (4 vs. 0). Across the entire trial, there were no serious study drug-related adverse events. The authors highlighted the promising nature of this approach, noting that the trial continues to enroll patients for further evaluation.[50]

Neoadjuvant ICI therapy has also been combined with chemotherapy or other systemic treatment. Toripalimab (a PD-1 inhibitor) in combination with albumin-bound paclitaxel/cisplatin (TTP) was evaluated in a single-arm prospective study (Illuminate Trial) in patients with locally advanced resectable oral squamous cell carcinoma (OSCC).[51] The protocol enrolled 20 patients with clinical stage III or IVA OSCC, who received 2 cycles of chemoimmunotherapy followed by radical surgery and risk-adapted adjuvant (chemo)radiotherapy. All patients underwent microscopically radical surgical procedures (R0) with a low incidence of significant adverse events during neoadjuvant therapy (only 3 patients with grade 3 or 4 events). Major pathological responses (MPRs) were observed in 60% of the clinical group, including 30% with

pCR. A favorable clinical response was associated with positive PD-L1 expression (>10%). The DFS rate was 90% and the OS rate was 95% after 26 months of follow-up.[51]

In another single-arm phase II trial, neoadjuvant therapy with 3 cycles of paclitaxel, cisplatin and toripalimab was tested in 27 patients with locally advanced laryngeal/hypopharyngeal squamous cell carcinoma.[52] After neoadjuvant therapy, participants with a complete or partial response of the primary tumor received concurrent chemoradiation followed by maintenance toripalimab. In other cases, patients underwent surgery followed by adjuvant chemoradiation and maintenance toripalimab. The primary endpoint was the larynx preservation rate at 3 months post-radiation. The overall response rate (ORR) was 85.2%, with an 88.9% post-radiation larynx preservation rate. After 1 year of follow-up, the OS rate was 84.7%, the progression-free survival (PFS) rate was 77.6%, and the larynx preservation rate was 88.7%.[52]

Despite some promising results in clinical trials, neoadjuvant therapy has not yet been incorporated into clinical practice based on the guidelines published by the European Society for Medical Oncology (ESMO)[53] and the National Comprehensive Cancer Network (NCCN).[54] The comparison of clinical trials focused on the neoadjuvant therapy for SCCHN is presented in Table 1.

Adjuvant immunotherapy after definitive surgery

Definitive surgery, alongside definitive radiotherapy, remains a primary therapeutic modality for head and neck cancers. In many cases, even after radical procedures, patients require adjuvant radiotherapy or chemoradiotherapy.[55] Adjuvant ICI therapy represents a potential strategy to improve prognosis, prolong DFS and provide an alternative option for platinum-ineligible patients requiring adjuvant treatment.

In an open-label, multi-institutional phase II clinical trial, patients with recurrent, resectable SCCHN received 6 adjuvant nivolumab cycles after salvage surgery.[56] Adjuvant nivolumab following salvage surgery was well-tolerated and demonstrated improved DFS as compared to historical controls. There was no significant difference in DFS between PD-L1-positive and PD-L1-negative patients; however, there was a non-significant trend toward improved DFS in patients with high tumor mutational burden ($p = 0.083$).[56]

In another phase II trial (ADJORL1), patients with recurrent SCCHN or second primary tumors in the previously irradiated areas underwent surgery with curative intent, followed by adjuvant nivolumab for 6 months.[57] A 2-year DFS was 46.6%, and a 2-year OS was 67.3%. Severe adverse events were reported in 19% of participants. The authors concluded that the 2-year DFS and OS outcomes were favorable when compared with historical data from reirradiation trials.[57]

In the PATHWay trial, high-risk SCCHN patients who had completed definitive treatment received adjuvant pembrolizumab therapy for 1 year.[58] ICI therapy improved PFS in 2 subgroups: post-salvage surgery patients (*HR* (hazard ratio): 0.34; 80% *CI* (confidence interval): 0.18–0.67; $p = 0.016$); and those with multiple recurrences/primaries (*HR*: 0.48; 80% *CI*: 0.27–0.88; $p = 0.057$). Severe adverse events were noted in 6% of participants.[58]

Based on these studies, there appears to be a potential role for ICI therapy in the adjuvant setting after definitive surgery; however, larger, multi-center clinical trials are necessary to confirm these findings. The comparison of clinical trials focused on the adjuvant therapy for SCCHN is presented in Table 2.

Table 2. Clinical trials focusing on adjuvant therapy for squamous cell carcinoma of the head and neck (SCCHN)

Trial identifier	Clinical phase	Tested agent(s)	Key outcomes
NCT03355560	II	nivolumab	improved DFS vs. historical controls
ADJORL1	II	nivolumab	2-year DFS: 46.6%, 2-year OS: 67.3%
PATHWay	II	pembrolizumab	improved PFS in post-salvage surgery and multiple recurrence patients

DFS – disease-free survival; OS – overall survival; PFS – progression-free survival.

Table 1. Clinical trials focusing on neoadjuvant therapy for squamous cell carcinoma of the head and neck (SCCHN)

Trial identifier	Clinical phase	Tested agent(s)	Key outcomes
NCT02296684	II	pembrolizumab	45% substantial pTR
CheckMate 358	I/II	nivolumab	12.0% ORR (HPV+) 8.3% ORR (HPV–)
–	II	durvalumab ± tremelimumab	improved DRFS with combination
NCT04080804	II	ipilimumab + nivolumab/relatlimab + nivolumab	higher pTR with doublet therapy
Illuminate	II	toripalimab + chemotherapy	60% MPR, 30% pCR
INSIGHT	II	toripalimab + chemotherapy	85.2% ORR, 88.9% larynx preservation rate

pTR – pathological tumor response; ORR – overall response rate; HPV – human papillomavirus; DRFS – distant recurrence-free survival; MPR – major pathological response; pCR – pathological complete response.

Immunotherapy in combination with definitive (radical) radiotherapy

Radiation therapy (RT) is an established method for both radical and palliative management of head and neck cancer. Radiation therapy can be administered alone, concomitantly/concurrently, or sequentially after induction chemotherapy. The most common technique is intensity-modulated radiation therapy (IMRT) using contemporary computer-based planning and radiation delivery with or without simultaneous integrated boost (SIB). Radiation therapy may also be considered as adjuvant therapy (with or without chemotherapy) after primary surgical treatment, or in cases where surgery could be harmful or unacceptable to the patient, and for the functional preservation of critical structures such as the larynx.[59,60]

The current standard of care includes the enhancement of standard RT with concomitant therapy, such as weekly cisplatin and platinum combined with 5-fluorouracil[61,62] or cetuximab.[63–66] Induction chemotherapy followed by concurrent chemoradiotherapy may also be considered in cases of advanced locoregional disease.[67] Despite the availability of multiple clinical options, novel therapeutic approaches could potentially improve prognosis and treatment outcomes. One of the most promising strategies is the application of ICIs before concomitant or concurrent therapy (the neoadjuvant approach).

The phase III JAVELIN Head and Neck 100 trial evaluated adjuvant 12-month avelumab therapy vs. placebo in 697 patients with locally advanced head and neck cancer after definitive cisplatin-based chemoradiotherapy.[68] The trial was terminated prematurely, as the boundary for futility had been crossed. The initial results showed a *HR* of 1.21 (95% *CI*: 0.93–1.57) and 1.31 (95% *CI*: 0.93–1.85) for PFS and OS, respectively.[68]

Another trial with avelumab, GORTEC-REACH, included 2 patient populations: cisplatin-fit patients who received standard-of-care cisplatin-based chemoradiation; and cisplatin-unfit patients who received weekly cetuximab and avelumab concurrently with radiation.[69] Both treatment regimens were followed by avelumab for 12 months vs. the standard of care. This trial was also negative, as the primary endpoint of improved PFS was not met for either cohort. The PFS *HR* was 1.27 (95% *CI*: 0.83–1.93) for the cisplatin-fit cohort, and the PFS *HR* at 2 years was 0.85 ($p = 0.15$) for the cisplatin-unfit cohort.[69]

In the KEYNOTE-412 study, adjuvant pembrolizumab added after concurrent cisplatin-based chemoradiotherapy was compared to placebo in 804 patients from 130 medical centers.[70] Although the trial showed a favorable trend, there was no statistically significant benefit for the pembrolizumab arm (*HR*: 0.83; 95% *CI*: 0.68–1.03). Even in the subpopulation with high PD-L1 expression (CPS ≥ 20), neither the median PFS nor OS were reached in either arm. The investigators reported neutropenia, stomatitis, anemia, dysphagia, lymphopenia, pneumonia, acute kidney injury, and febrile neutropenia as significant adverse events. The authors concluded that the addition of pembrolizumab to chemoradiotherapy did not significantly improve event-free survival (EFS) as compared to placebo in a molecularly unselected locally advanced SCCHN population.[70]

The preliminary results of maintenance nivolumab therapy following definitive chemoradiotherapy showed an encouraging safety profile and some significant improvement in OS and PFS for patients with intermediate-risk HPV-positive oropharyngeal cancer that had spread to nearby tissue or lymph nodes. However, phase III of the EA3161 trial is ongoing.[71] Another ongoing trial, NRG-HN005, will evaluate the effectiveness and safety of de-intensified radiation therapy in combination with cisplatin or immunotherapy with nivolumab in patients with early-stage, HPV-positive, non-smoking-associated oropharyngeal cancer.[72]

ICI therapy can also be administered as adjuvant treatment after definitive radiotherapy, as demonstrated in the phase III IMvoke010 trial with atezolizumab.[73] This study included 406 patients with locally advanced SCCHN (stage IVa or IVb) without disease progression after radical chemoradiotherapy. Participants were randomized to receive 1 year of atezolizumab or placebo. The trial was negative, showing no difference in OS between the arms.[73] In contrast, the phase III NIVOPOSTOP GORTEC 2018-01 trial demonstrated statistically significant improvement in DFS in the nivolumab arm as compared to placebo after definitive chemoradiotherapy, although complete data presentation is still pending.[74] A trial testing the combination of atezolizumab with cetuximab after chemoradiotherapy in high-risk head and neck cancer is currently enrolling participants.[75] Another approach combines neoadjuvant radiotherapy with ICIs before radical surgical resection.[76] In the phase II KEYNOTE-689 trial, neoadjuvant pembrolizumab was followed by surgical tumor ablation, and subsequently by postoperative (chemo)radiation. Furthermore, participants with high-risk pathology (positive margins and/or extranodal extension) received adjuvant pembrolizumab. Results were presented as pTR: pTR-0 < 10%; pTR-1 10–49%; and pTR-2 ≥ 50%. From the entire study population, 22% of patients had pTR-1, 22% had pTR-2, and none had pTR-3. After 1 year, 16.7% of participants with high-risk pathology experienced disease relapse.[76] The phase III IMSTAR-HN trial, evaluating nivolumab monotherapy or combined with ipilimumab vs. the standard of care in resectable SCCHN,[77] and the CompARE trial with durvalumab in patients with intermediate and high-risk oropharyngeal cancer[78] are currently randomizing participants. The comparison of clinical trials focused on immunotherapy in combination with definitive (radical) radiotherapy for SCCHN is presented in Table 3.

Table 3. Clinical trials focusing on immunotherapy in combination with definitive (radical) radiotherapy for squamous cell carcinoma of the head and neck (SCCHN)

Trial identifier	Clinical phase	Tested agent(s)	Key outcomes
JAVELIN Head and Neck 100	III	avelumab	negative for PFS and OS improvement
GORTEC-REACH	III	avelumab	negative for PFS improvement
KEYNOTE-412	III	pembrolizumab	negative for EFS improvement
EA3161	II/III	nivolumab	ongoing preliminary positive signal
NRG-HN005	III	nivolumab	ongoing
IMvoke010	III	atezolizumab	negative for OS improvement
NIVOPOSTOP GORTEC 2018-01	III	nivolumab	significant DFS improvement (full data pending)
KEYNOTE-689	II	pembrolizumab	22% pTR-1, 22% pTR-2, 16.7% relapse at 1 year in high-risk patients
IMSTAR-HN	III	nivolumab ± ipilimumab	ongoing
CompARE	II	durvalumab	ongoing

EFS – event-free survival.

ICI therapy in metastatic or relapsed head and neck cancer

ICI therapy in the treatment of recurrent/metastatic (R/N) head and neck cancer has established a position in routine clinical practice, as confirmed by the guidelines of ESMO[53] and NCCN.[54] Multiple clinical trials have led to the routine evaluation of PD-L1 expression through CPS, defined as the number of PD-L1-positive cells (tumor cells, lymphocytes and macrophages) divided by the total number of tumor cells, multiplied by 100.

The initial investigation was the phase Ib KEYNOTE-012 trial, which first suggested the manageable toxicity and promising anti-tumor activity of pembrolizumab in patients with R/N SCCHN.[79] Subsequently, the single-arm phase II KEYNOTE-055 study evaluated pembrolizumab therapy in 171 patients (CPS ≥ 50 in 48 patients) with R/N SCCHN refractory to platinum-based therapy and cetuximab.[80] Of these patients, 82% were PD-L1-positive and 22% were HPV-positive. The ORR was 16%, with a median duration of response (DoR) of 8 months. The median PFS was 2.1 months, and OS was 8 months. Adverse events occurred in 64% of patients, but only 15% experienced grade 3 or higher events, with fatigue, hypothyroidism, nausea, and increased aspartate aminotransferase (AST) being most common. Statistical analysis revealed that HPV-positive patients demonstrated higher 6-month OS (72%) as compared to 55% in the HPV-negative subgroup; however, ORR and PFS were similar. The ORR was associated with the PD-L1 expression status (18% for CPS ≥ 1 and 27% for CPS ≥ 50).[80]

Following these preliminary findings, pembrolizumab demonstrated its value in the phase III KEYNOTE-048 trial.[81] According to the protocol, participants were randomized to 3 arms: pembrolizumab monotherapy; pembrolizumab with platinum and 5-fluorouracil; or cetuximab with platinum and 5-fluorouracil (standard of care). Statistical analysis was stratified by PD-L1 expression defined by CPS. In the population with CPS ≥ 20, pembrolizumab monotherapy was associated with improved median OS as compared to cetuximab with chemotherapy (14.9 months vs. 10.7 months, *HR*: 0.61; 95% *CI*: 0.45–0.83; $p = 0.0007$). Furthermore, pembrolizumab with chemotherapy improved OS vs. cetuximab with chemotherapy in the total population (13.0 months vs. 10.7 months, *HR*: 0.77; 95% *CI*: 0.63–0.93; $p = 0.0034$), irrespective of CPS. The final analysis showed 2 populations that benefited from pembrolizumab therapy: those with CPS ≥ 20 (14.7 months vs. 11.0 months, *HR*: 0.60; 95% *CI*: 0.45–0.82; $p = 0.0004$); and those with CPS ≥ 1 (13.6 months vs. 10.4 months, *HR*: 0.65; 95% *CI*: 0.53–0.80; $p < 0.0001$). Despite these survival benefits, neither pembrolizumab alone nor pembrolizumab with chemotherapy improved PFS. Severe adverse events were reported in 55% of the pembrolizumab monotherapy arm, 85% of the pembrolizumab with chemotherapy arm, and 83% of the cetuximab with chemotherapy group.[81] The KEYNOTE-048 trial led to change in the standard of care, and pembrolizumab in monotherapy or in combination with chemotherapy are now accepted regimens in the therapy of PD-L1-positive (CPS ≥ 1) R/N SCCHN.[53,54]

Nivolumab was approved for the second-line treatment of platinum-refractory R/N SCCHN based on the phase III CheckMate 141 trial.[82] Ferris et al. enrolled 361 subjects who were randomized to receive either nivolumab or standard treatment (methotrexate, docetaxel or cetuximab). The nivolumab arm demonstrated higher median OS (7.5 months vs. 5.1 months) with a better safety profile (severe adverse events: 13.1% vs. 35.1%), irrespective of PD-L1 expression (<1% or ≥1%).[82]

Durvalumab therapy in a phase Ib/IIa study of immunotherapy-naive patients who had previously received platinum-containing regimens was well-tolerated[83] and led to further clinical trials. For example, the phase II HAWK

study focused on a population with high PD-L1 expression (≥25%) with platinum-refractory R/N SCCHN.[84] Patients received durvalumab monotherapy for up to 12 months. The median PFS and OS were 2.1 months and 7.1 months, respectively. At the endpoint, the PFS and OS rates were 14.6% (95% *CI*: 8.5–22.1) and 33.6% (95% *CI*: 24.8–42.7), respectively. Severe adverse events were noted in 8.0% of patients. The authors concluded that durvalumab demonstrated anti-tumor activity with acceptable safety in PD-L1-high patients with R/N SCCHN, although further phase III trials are needed. The subsequent analysis showed higher ORR (29.4% vs. 10.9%) and longer OS (10.2 months vs. 5.0 months) with durvalumab in HPV-positive patients.[84]

The addition of tremelimumab (anti-CTLA-4) to durvalumab therapy in the phase II CONDOR study[85] and in the phase III randomized open-label EAGLE study[86] did not demonstrate significant differences in ORR, OS, PFS, or DoR in patients with R/N SCCHN. Similarly, the CheckMate 651 trial compared ipilimumab plus nivolumab to cetuximab plus cisplatin/carboplatin plus fluorouracil (EXTREME regimen) followed by cetuximab maintenance in the first-line therapy of R/N SCCHN.[87] This study was also negative, with no statistically significant differences in the median OS in the total population (13.9 months vs. 13.5 months, *HR*: 0.95; 97.9% *CI*: 0.80–1.13; $p = 0.4951$) or in the CPS ≥ 20 population (17.6 months vs. 14.6 months, *HR*: 0.78; 97.51% *CI*: 0.59–1.03; $p = 0.0469$). The PFS (5.4 months vs. 7.0 months) and ORR (34.1% vs. 36.0%) were also similar between the treatment arms.[87]

The combination of ipilimumab and nivolumab was further tested in CheckMate 714 for the treatment of R/N SCCHN.[88] Participants were randomized 2:1 to receive nivolumab plus ipilimumab or nivolumab plus placebo for up to 2 years or until disease progression, unacceptable toxicity or consent withdrawal. The ORR for platinum-refractory therapy in the doublet therapy arm was 13.2% (95% *CI*: 8.4–19.5) as compared to 18.3% in the monotherapy arm (95% *CI*: 10.6–28.4) ($p = 0.290$). The median DoR for nivolumab plus ipilimumab was not reached vs. 11.1 months for nivolumab alone. In patients with platinum-eligible disease, ORRs were 20.3% vs. 29.5%. The incidence of severe adverse events was similar – 15.8% for ipilimumab-nivolumab vs. 14.6% for nivolumab. The study did not meet its primary endpoint of demonstrating an ORR benefit with first-line ipilimumab-nivolumab therapy in platinum-refractory R/M SCCHN.[88]

Another ICI, avelumab, was evaluated in the JAVELIN Solid Tumor phase Ib trial in patients with platinum-refractory/ineligible R/M SCCHN, and demonstrated safety and modest clinical activity.[89]

The comparison of clinical trials focused on immunotherapy for metastatic or relapsed SCCHN is presented in Table 4.

Future perspectives

ICI therapy in head and neck cancer represents a promising frontier in improving patient outcomes. Recent advancement points to several emerging strategies that may enhance the efficacy of current approaches. The phase III LEAP-010 study evaluated pembrolizumab and lenvatinib (anti-LAG) for R/N SCCHN.[90] Patients were randomized to receive either pembrolizumab 200 mg plus placebo (control) or pembrolizumab plus lenvatinib 20 mg daily (experimental group). Treatment continued for up to 35 cycles or until intolerable toxicity, progression or withdrawal. The median PFS (6.2 months vs. 2.8 months, $p = 0.0001040$) and ORR (46.1% vs. 25.4%, $p = 0.0000251$) were significantly improved in the experimental arm at the first interim analysis. However, the second interim analysis showed no significant difference in the median OS (15.0 months vs. 17.9 months, $p = 0.882$). The rate of severe adverse events was higher in the experimental arm (28% vs. 8%).[90]

Table 4. Clinical trials focusing on immunotherapy for metastatic or relapsed squamous cell carcinoma of the head and neck (SCCHN)

Trial identifier	Clinical phase	Tested agent(s)	Key outcomes
KEYNOTE-012	Ib	pembrolizumab	manageable toxicity, promising activity
KEYNOTE-055	II	pembrolizumab	ORR: 16%, median OS: 8 months
KEYNOTE-048	III	pembrolizumab ± chemotherapy	improved OS in CPS ≥ 1 and CPS ≥ 20
CheckMate 141	III	nivolumab	improved OS vs. standard therapy (7.5 months vs. 5.1 months)
HAWK	II	durvalumab	median OS: 7.1 months in PD-L1-high patients
CONDOR	II	tremelimumab + durvalumab	no significant benefit over monotherapy
EAGLE	III	tremelimumab + durvalumab	no significant benefit over monotherapy
CheckMate 651	III	ipilimumab + nivolumab	no OS advantage over the EXTREME regimen
CheckMate 714	II	ipilimumab ± nivolumab	no ORR advantage with combination
JAVELIN Solid Tumor	Ib	avelumab	demonstrated safety, modest activity

CPS – combined positive score; PD-L1 – programmed death ligand-1.

The phase II LEAP-009 study demonstrated promising efficacy and safety for the lenvatinib and pembrolizumab combination in R/M SCCHN, which progressed after platinum and immunotherapy.[91] Other potential enhancers of ICI therapy in the first-line R/M SCCHN treatment include the poly(ADP-ribose) polymerase (PARP) inhibitor olaparib,[92] the bifunctional EGFR/tumor growth factor beta (TGF-β) inhibitor BCA101,[93] the multi-kinase inhibitor zanzalintinib,[94] and recombinant IL-2 bempegaldesleukin.[95] Collectively, these findings point to the next generation of clinical trials in SCCHN, focusing on combining targeted therapies with ICIs.

Improved patient outcomes may also result from the neoadjuvant applications of immune checkpoint blockade (ICB). Recent studies suggest that applying ICB in the neoadjuvant setting could potentially promote systemic anti-tumor immunity, although further research is needed.[96] Combination therapy with other immune-stimulating molecules often yields more successful outcomes than PD-1 inhibitor monotherapy in SCCHN. A recent systematic review and meta-analysis of 7 phase I, II and III trials revealed that combination therapy significantly improved ORR and 1-year OS in HPV-negative R/N SCCHN as compared to anti-PD-1 monotherapy; however, this benefit was not observed in HPV-positive cases.[97]

The indoleamine 2,3-dioxygenase-1 (IDO1) inhibitor epacadostat is still being investigated for potential PFS benefits when combined with pembrolizumab.[98] Previously, epacadostat was evaluated in combination with PD-1 inhibitors in advanced solid tumors, demonstrating a tolerable safety profile and a relatively high ORR in the ECHO-304/KEYNOTE-669 study.[99] Another IDO1 inhibitor, navoximod, was tested in combination with atezolizumab in a phase I trial for patients with solid tumors, showing a favorable safety profile, but inconclusive efficacy results.[100]

According to the SCORES study, the STAT3 inhibitor danvatirsen (AZD9150) demonstrated safety for use in combination with PD-1 inhibitors.[101] The study also indicated potential anti-tumor activity of ICIs, with additional trials currently in progress.[102–104] The most promising future directions are presented in Fig. 1.

An inherent limitation of our review is the presence of ongoing clinical trials that may ultimately alter the paradigm of systemic therapy for head and neck cancer. Although several of these studies are discussed above, many currently report only partial or interim results.

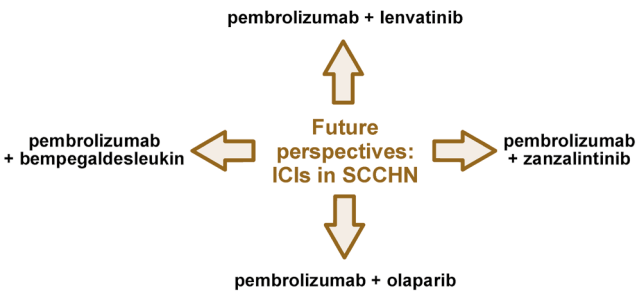

Fig. 1. Immune checkpoint inhibitors (ICIs) for squamous cell carcinoma of the head and neck (SCCHN): Future perspectives

Conclusions

Immunotherapy has been integrated into everyday clinical practice for metastatic or relapsed SCCHN, as confirmed by the ESMO and NCCN guidelines.[53,54] Other applications of ICIs in the management of SCCHN remain under development, with ongoing research focused on optimizing their efficacy and safety. In contrast, the inhibitors of the PD-1/PD-L1 pathway – most notably pembrolizumab and nivolumab – have demonstrated substantial clinical benefit in R/M SCCHN, and have therefore been incorporated into standard treatment algorithms. The role of CTLA-4 inhibitors, whether as monotherapy or in combination with PD-1/PD-L1 inhibitors, is less well established, but remains an active area of investigation.

The neoadjuvant and adjuvant use of ICIs has shown encouraging preliminary results in early-phase trials; however, larger randomized studies are required before these strategies can be adopted into routine clinical practice. Similarly, the combination of ICIs with definitive radiotherapy has produced mixed outcomes, with some trials demonstrating benefits, while others have failed to meet their primary endpoints.

Future directions in the field include the development of novel combination strategies incorporating targeted therapies, the identification of predictive biomarkers beyond PD-L1 expression, the design of immunotherapy approaches tailored to HPV-positive vs. HPV-negative disease, and the optimization of treatment sequencing and duration. As understanding of tumor immunology and the mechanisms underlying response and resistance to immunotherapy continues to advance, the therapeutic landscape for SCCHN is expected to further evolve, with the potential to improve outcomes in this challenging disease.[105]

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

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Use of AI and AI-assisted technologies

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Research letter

Analysis of selected parameters of reparative dentin after direct pulp capping with MTA Repair HP in human teeth, using CBCT and micro-CT

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Conflict of interest
None declared

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Abstract

Background. The biomimetic regeneration of the pulp–dentin complex is a modern approach involving the application of a bioactive substance. Bioactive substances (e.g., cement or stem cells) have been reported to be able to control the signaling and differentiation of the pulp cells, thus limiting the inflammatory reactions and enabling the repair and regeneration of tissues. One of such materials is MTA Repair HP (MTA HP) – a type of mineral trioxide aggregate (MTA) material characterized by high plasticity, composed of hydrophilic particles of mineral oxides.

Objectives. The aim of the present study was to analyze the reaction of the pulp–dentin complex of human teeth to MTA HP using 2 radiological techniques – cone-beam computed tomography (CBCT) and micro-computed tomography (micro-CT).

Material and methods. In the study, 6 caries-free, intact maxillary and mandibular third molars or premolars from 6 patients aged 30–37 years were analyzed. The teeth were scheduled to be extracted for orthodontic or surgical reasons.

Results. No statistically significant differences in the examined parameters of the tertiary dentin were observed in the CBCT and micro-CT images ($p < 0.05$).

Conclusions. The CBCT technique, similarly to micro-CT, proved to be effective in identifying and assessing the tertiary dentin. Furthermore, CBCT has the advantage of usability in clinical settings. Knowledge on the parameters of reparative dentin identified with radiological techniques is still insufficient, and further research is recommended.

Keywords: micro-CT, CBCT, MTA HP, biomimetic material, reparative dentin

Highlights

- The biomimetic regeneration of the pulp–dentin complex is a modern approach involving the application of a bioactive substance, such as mineral trioxide aggregate (MTA).
- After applying MTA Repair HP to human teeth (maxillary and mandibular third molars or premolars), no statistically significant differences in the examined parameters of the tertiary dentin were observed in the CBCT and micro-CT images.
- The CBCT technique, similarly to micro-CT, proved to be effective in identifying and assessing the tertiary dentin.

Introduction

Vital pulp therapy (VPT) is part of the minimally invasive endodontics.[1–3] At the molecular level, in the context of supporting wound healing and the regeneration of soft and hard tissues, the application of biomimetic materials onto the pulp–dentin complex has been widely studied, as these materials have a biostimulating effect on the regenerative processes of the pulp–dentin complex.[4]

Apart from clinical examinations, cone-beam computed tomography (CBCT) examinations are used to assess the success of VPT. However, in comparison with CBCT, micro-computed tomography (micro-CT) allows imaging with improved parameters of given structures and fewer image distortions. At the same time, in comparison with the most frequently applied histological techniques, micro-CT does not result in sample destruction, so the material can be analyzed repeatedly. The literature on the subject encompasses visual analyses and quantitative assessments of the newly formed reparative dentin, following the use of various bioactive cements, using CBCT and micro-CT. In human teeth, reparative dentin has been analyzed with the CBCT and micro-CT techniques.[5–10] Micro-CT has also been used for visualizing reparative dentin in the teeth of rats, mice and old baboons.[11–16]

Various biomimetic preparations are used in VPT, all aimed at supporting natural defensive and regenerative mechanisms, and the eventual healing of pathological changes.[17] One of such materials is MTA Repair HP (MTA HP). Mineral trioxide aggregate (MTA) has a wide range of applications in endodontics, i.a., for direct pulp capping (DPC), closing root canal perforations, as well as the perforations which occur within the furcation regions due to iatrogenic reasons, treating carious lesions or resorption, and retrograde root canal filling within periapical surgery.[18–21]

The aim of the present study was to analyze the reaction of the pulp–dentin complex of human teeth to the direct application of MTA HP, using 2 radiological techniques – CBCT and micro-CT. The study used both techniques to assess the structural parameters of the reactionary dentin (the dentin bridge) formed underneath the MTA HP material in human teeth. The null hypothesis states that the CBCT and micro-CT techniques provide comparable imaging of reparative dentin structures in human teeth.

Material and methods

Material

In the study, 6 caries-free, intact maxillary and mandibular third molars or premolars from 6 patients aged 30–37 years were analyzed. The teeth were scheduled to be extracted for orthodontic or surgical reasons. The study was conducted in full accordance with ethical principles, including the World Medical Association (WMA) Declaration of Helsinki (2008). The patients received a thorough explanation of the experimental rationale, clinical procedures and possible complications. The experiments were undertaken with the understanding and written consent of each patient, and according to the abovementioned principles. All experimental protocols were independently reviewed and approved by the Local Ethics Committee of the Pomeranian Medical University in Szczecin, Poland (approval No.: KB/2020/47/NK).

Operating procedure

Periapical radiographs were taken for all the analyzed teeth (MINRAY®, Soredex, Tuusula, Finland). Tooth sensitivity was assessed with thermal testing (Kältespray; M+W Dental, Büdingen, Germany) and electric sensitivity testing (Vitality Scanner Electric Pulp Tester; SybronEndo, Orange, USA). All clinical procedures were performed by the same clinician, who used an operating microscope with ×4 magnification (OPMI Pico; Carl Zeiss, Jena, Germany). Occlusal class I on the occlusal surfaces was prepared with a sterile round diamond bur at a high speed, with standard abundant water cooling. When approaching the pulp chamber, a new sterile round diamond bur (ISO size 012) was used to gently produce an exposure of at least 1 mm^2. Bleeding was controlled by irrigation with a saline solution (0.9% NaCl) and a sterile cotton pellet. The exposed pulp was capped with a 2–3-millimeter-thick layer of MTA HP (MTA Repair HP; Angelus USA Inc., Vernon, USA). MTA-HP was covered with a thin layer of glass ionomer (Riva Light Cure HV; SDI, Chicago, USA). Finally, the cavity was filled using Single Bond™ Universal with the self-etch technique and Filtek™ Ultimate (3M ESPE, Seefeld, Germany).

Clinical examination

The evaluation of the pulp status was conducted based on patient-reported symptoms with a verbal pain intensity scale, clinical examinations, and thermal and electrical tests. Immediately after cavity restoration, the patients were asked to register their level of discomfort. Clinical assessment was performed 6 weeks after the procedure.

Radiological examination

Six weeks after DPC with MTA-HP, before extraction, periapical radiographs were taken (DIGORA™ Optime UV; Soredex) using the right angle technique with positioners. The identical exposure parameters were applied in all cases. Subsequently, the teeth were extracted as atraumatically as possible by a qualified oral surgeon under local anesthesia with mepivacaine without a vasoconstrictor (Scandonest®, Septodont, Saint-Maur-des-Fossés, France). The CBCT and micro-CT examinations were performed after tooth extraction. Each tooth was scanned separately, using the following exposure parameters: source voltage [kV]: 60–70 automatic control (CBCT), 90 (micro-CT); source amperage [μA]: 100–750 automatic control (CBCT), 111 (micro-CT); resolution [pixels]: 341 × 341 (CBCT), 4,032 × 2,688 (micro-CT); pixel size [μm]: 500 (CBCT), 4.5 (micro-CT); exposure time [ms]: 5,500 (CBCT), 1,306 (micro-CT); and filter: Cu 0.2 (CBCT), Al 0.5 + Cu 0.038 (micro-CT). In the CBCT analysis of a given tooth, reparative dentin was identified in original axial areas and in multidimensional reconstructions with the Veraview IC5 HD tomograph (J. Morita, Ina-machi, Japan). The three-dimensional (3D) image analysis procedures were performed using the open-source software ITK-SNAP, v. 3.8 (http://www.itksnap.org/pmwiki/pmwiki.php) and 3D Slicer (SlicerCMF extension of 3D Slicer, v. 4.11.0; https://www.slicer.org). The original de-identified DICOM files were converted to nii.gz files using ITK-SNAP.[22] In the micro-CT analysis of a given tooth, the identification of reparative dentin was performed using SKYSCAN 1272 (Bruker Corporation, Kontich, Belgium), and the 3D reconstructions of the teeth were obtained using the NRecon 1.7.4.2 and CTvox 3.3.0 r1403 software by Bruker. The geometrical parameters of the tested reparative dentin were calculated using the CTAn 1.17.7.2+ software by Bruker.

In both the CBCT and micro-CT analyses, the region of the exposed pulp was identified as the drilled area of the pulp chamber roof. The largest diameter of the exposed pulp was calculated (in millimeters).

Identification of reparative dentin

In both the CBCT and micro-CT examinations, the area of the tertiary dentin was determined entirely manually. This was due to the low contrast between the tertiary and secondary dentin. Two-dimensional (2D) sections were obtained in the transverse plane to determine the first and the last layer in the crown-apical direction so that reparative dentin could be identified. In comparison with the primary dentin, it was assumed that the calcified region underneath the pulp-capping material is the region of the induced tertiary dentin. The tertiary dentin was marked green, using special software (SKYSCAN 1272) with a tool for coloring the regions of interest (ROI).

The visual assessment of reparative dentin was conducted through the micro-CT analysis in 3 planes normalized with the color change scale. Following the reconstruction of the images, grey areas were displaced by color, taking advantage of the fact that the areas of correct pulp, dentin and enamel are X-ray-permeable to different extent. It was adopted that in a correctly formed tooth, the green and blue colors represent fillings, and purple represents pulp. Therefore, reparative dentin was defined as orange, and different color saturation of the tissue was considered as differences in calcification as compared to physiological dentin. The 3D reconstructions of the tested teeth were obtained using the NRecon 1.7.4.2 and CTvox 3.3.0 r1403 software.

Quantitative assessment of reparative dentin

Thickness of reparative dentin

The reparative dentin thickness was determined using a linear measurement tool and measuring the distance from the base of the restoration to pulp in the sagittal, coronal or cross-sectional images generated from the CBCT or micro-CT scans after the orientation of the orthogonal planes with the use of the software vertical and horizontal reference lines.[23] The serial profile of reparative dentin formed in the direction from the crown (the first virtual layer) to the root (the last virtual cross-section) was determined using tooth axial layers in the CBCT analysis size 0.125 mm, whereas in the micro-CT analysis, the size was 0.0045 mm; it allowed the calculation of the estimated thickness of the tertiary dentin (in millimeters).

Volume of reparative dentin

A solid figure was isolated from the obtained images of all planes, corresponding to the formed tertiary dentin; its volume (in cubic millimeters) was mathematically calculated in the CBCT analysis using ITK-SNAP, v. 3.8., and in the micro-CT analysis, the CTAn 1.17.7.2+ software by Bruker.

Statistical analysis

The mean (*M*), standard deviation (*SD*), median (*Me*), minimum (min), and maximum (max) values, and the coefficient of variation (*CV*) were calculated for the thickness and volume of the tertiary dentin, and the diameter of the exposed pulp. The distribution of values deviated

from normal distribution in the Shapiro–Wilk test. Therefore, the non-parametric Mann–Whitney test was used to compare the variables.

Results

Clinical examination

All the analyzed teeth were characterized by a normal sensitivity response to a cold stimulus (pain lasting up to 10 s). Sensitivity to electric current was within the normal values in the range of 23–47 units. None of the patients reported pain after treatment.

Radiological examination

Based on the X-ray images, it was found that in all teeth under study there was a lack of pathological lesions, such as the presence of mineralization in the pulp chamber, or internal or external resorption, which would suggest the failure of DPC.

Identification and visual assessment of reparative dentin

In all teeth under analysis, the presence of reparative dentin was identified. The visual assessment of reparative dentin in the CBCT and micro-CT analyses was performed in 2D and 3D projections. The data concerning each sample is presented in Fig. 1, comparing the X-ray, CBCT and micro-CT images of tooth 18 after covering pulp with the MTA HP material.

Quantitative assessment of reparative dentin

Table 1 shows the descriptive statistics of the selected geometrical parameters of reparative dentin and the pulp exposure in CBCT and micro-CT. Concerning the geometric parameters of reparative dentin, it was found that the mean thickness and volume of reparative dentin were 0.39 ±0.24 mm and 0.42 ±0.29 mm^3 in CBCT, and 0.31 ±0.18 mm and 0.30 ±0.22 mm^3 in micro-CT, respectively. There were no statistically significant differences in the

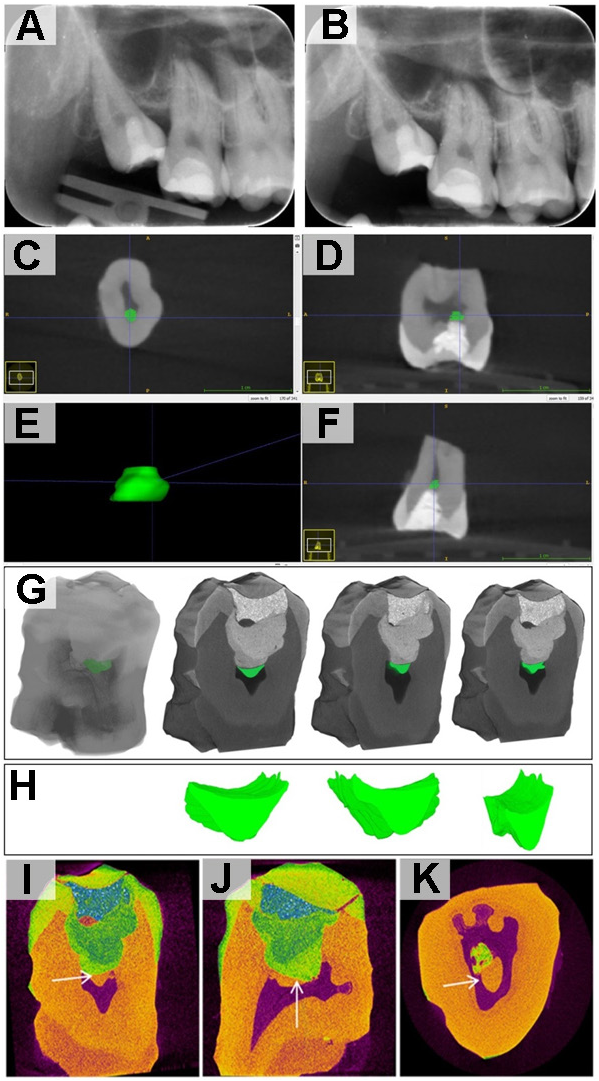

Fig. 1. Comparison of the X-ray, cone-beam computed tomography (CBCT) and micro-computed tomography (micro-CT) images of tooth 18 after covering pulp with the MTA (mineral trioxide aggregate) Repair HP (MTA HP) material

A – X-ray: tooth 18 equipped with the MTA HP material immediately after the direct pulp capping (DPC) procedure; B – X-ray: tooth 18 equipped with the MTA HP material 6 weeks after the DPC procedure; C–F – CBCT: tooth 18 equipped with the MTA HP material 6 weeks after the DPC procedure (green color – reparative dentin); G – micro-CT: tooth 18 equipped with the MTA HP material 6 weeks after the DPC procedure (green color – reparative dentin); H – micro-CT: tooth 18 – the isolated solid figure of reparative dentin; I–K – micro-CT: tooth 18 equipped with the MTA HP material 6 weeks after the DPC procedure (white arrows – reparative dentin, orange color – dentin, green and blue colors – filling, purple color – pulp).

Table 1. Comparison of the geometrical parameters of reparative dentin and the pulp exposure in CBCT and micro-CT

Parameter	Technique	*N*	*M* ±*SD*	*Me*	min	max	*CV*
Reparative dentin thickness [mm]	CBCT	6	0.39 ±0.24	0.32	0.16	0.78	61.32
	micro-CT	6	0.31 ±0.18	0.29	0.14	0.62	57.11
Reparative dentin volume [mm^3]	CBCT	6	0.42 ±0.29	0.42	0.10	0.87	70.11
	micro-CT	6	0.30 ±0.22	0.33	0.05	0.56	73.54
Pulp exposure diameter [mm]	CBCT	6	1.47 ±0.53	1.56	0.75	2.25	35.76
	micro-CT	6	1.48 ±0.56	1.27	0.89	2.36	38.16

M – mean; *SD* – standard deviation; *Me* – median; min – minimum; max – maximum; *CV* – coefficient of variation.

thickness and volume of the tertiary dentin, and in the diameter of the exposed pulp in CBCT and micro-CT ($p < 0.05$). A moderate correlation ($r = 0.67$), at the confidence level of 0.95, was marked between the pulp exposure values obtained with both techniques (CBCT vs. micro-CT). For CBCT, there was a strong positive correlation between the minimum and maximum thickness values ($r = 0.91$), and for micro-CT, the correlation between the values was very weak, expressed with $r = 0.48$. The correlation between the reparative dentin volume values in CBCT and micro-CT was very weak ($r = 0.43$).

Discussion

The formation of compact, hard reparative dentin without bacterial invasion is the crucial key to the success of VPT.[13,24] The average diameter of the exposed pulp in the examined teeth was 1.47 mm in the CBCT examination and 1.48 mm in the micro-CT examination. Statistical analysis showed no difference in the size of the exposed pulp with regard to the technique used. The obtained values are consistent with those mentioned by other authors.[5,8]

Reparative dentin is a highly heterogeneous structure. Isolating a whole solid is a complex and time-consuming endeavor. However, the parameters of reparative dentin have been calculated and discussed in various studies.[10–20,22,25] In all the analyzed teeth, the presence of the newly formed reparative dentin was identified through both CBCT and micro-CT; however, the tissue showed differences in terms of shape and size. Reparative dentin was formed in an irregular and unpredictable manner, though its form was closely related to the shape and size of the exposed pulp and the MTA HP material covering the wound. Therefore, it can be cautiously concluded that the actions of the operator conducting DPC affect the shape of the newly formed reparative dentin. With greater insertion of the material to the pulp wound, the reparative dentin bridge is more irregular and larger. More homogeneous reparative dentin, without defects such as a tunnel defect, provides better protection for the exposed pulp.

The visual analysis of the tertiary dentin with the use of CBCT relies on identifying the presence of islands of calcified tissue, as well as detecting complete (continuous) tertiary dentin.[5] Nowicka et al. used the CBCT technique to show the presence of 25 tertiary dentin bridges out of the 37 histologically identified bridges after the DPC of human teeth with various materials.[8] In another similar study on humans, 8 weeks after the application of various materials, the highest number of complete tertiary dentin bridges was recorded in the premolar group, following pulp capping with MTA and EndoSequence Root Repair Material (ERRM).[9]

The visual analysis of the parameters of reparative dentin is frequently carried out in micro-CT.[10,12,13,15,16] The analyses of reparative dentin morphology were most frequently conducted on animal teeth in the procedure of indirect or direct pulp capping with various MTA cements.[13,16,23–25] In a micro-CT based study on 10 molar rat teeth, after 4-week observations, the areas of the newly formed reparative dentin and the pulp cavity were measured in 5 randomly selected transverse sections, followed by the calculation of the relative ratio of the reparative dentin area to the pulp cavity area.[16] Following reconstruction, in order to show mineral density, grey images were displaced by color images. In visual assessment, greater development of the tertiary dentin was identified in the teeth capped with the MTA material.[16] In our study, similarly to the study by Kim et al.,[16] the color change scale was adopted using the NRecon 1.7.4.2 and CTvox 3.3.0 r1403 software.

Apart from the visual assessment of reparative dentin, the quantitative assessment of its physical parameters, such as thickness and volume, is important. In comparison with visual assessment, quantitative assessment is more precise and objective.

With regard to CBCT imaging, the thickness of reparative dentin has been analyzed in human teeth. In a study by Nowicka et al., the thickness of the tertiary dentin in the CBCT images was 0.23 mm in the MTA group.[8] In the present study, the mean thickness of the dentin bridge in the 6 analyzed samples was 0.39 mm. The difference could be due to the size of the evaluated samples and the measurement technique.

Kim et al. analyzed the thickness of reparative dentin with micro-CT in 30 premolar old baboon teeth, in which the exposed pulp was capped with calcium hydroxide ($Ca(OH)_2$), ProRoot® MTA White or white Portland cement (PC).[16] Following the use of ProRoot MTA White, the reparative hard tissue achieved a thickness of 0.43 ±0.05 mm.[16] AlShwaimi et al. analyzed the exposed pulp of 18 human premolar teeth, which were capped with betamethasone/gentamicin (BG) cream and MTA.[13] It was found that the thickest reparative dentin was formed in the teeth capped with MTA. The mean thickness of the reparative hard tissue was 0.078 mm.[13] The present study shows that the identified range of the thickness values 6 weeks after DPC with MTA HP was 0.14–0.78 mm. There was no statistically significant difference in the thickness of the tertiary dentin between the CBCT and micro-CT techniques. The mean thickness calculated based on the thickness value ranges of the individual 6 teeth under analysis was 0.39 mm, which is in line with the results obtained in the abovementioned studies. However, it should be noted that the calculation of the mean thickness of reparative dentin presented herein should be treated as a guidance value, since other factors must be taken into account as well, e.g., the number of samples (6–30), the type of teeth under analysis, study time (between 8 weeks and 4 months), species (humans, old baboons), and the material used for DPC.

Generally, determining the volume of reparative dentin is not possible using conventional radiovisiography, and is difficult using the CBCT and micro-CT techniques. A study using CBCT in the analysis of human teeth, 6 weeks after extraction demonstrated that the tomography resolution negatively affected the detection of the minimum measurable distance, and therefore histological images were necessary to measure very small bridges.[8] It was found that in the $Ca(OH)_2$ group, the volume of the formed reparative dentin was moderate, whereas in the MTA (0.45 mm^3) and Biodentine groups (0.47 mm^3), it was moderate to high, with no significant differences between the latter two groups.[8] In the present study, when employing the CBCT analysis, the mean volume of dentin bridges amounted to 0.42 mm^3, which is slightly lower than the results obtained by Nowicka et al.[8] The difference may result from different materials used in the studies.

In our study, the volume of reparative dentin in human teeth was measured with the micro-CT technique for the first time. To achieve the highest precision in the measurement of the geometrical parameters of the dentin bridge, the ROI enclosing the analyzed reparative dentin was determined manually. This principle was adopted in the present study with respect to both the CBCT and micro-CT techniques, and there was no statistically significant difference in the volume of the tertiary dentin between these techniques. CBCT, similarly to micro-CT, was effective in identifying and assessing tertiary dentin, so the null hypothesis was accepted. However, the limitation of the present study is the small number of samples, which was due to the difficulty in obtaining the study material.

Conclusions

The CBCT technique, similarly to micro-CT, proved to be effective in identifying and assessing the tertiary dentin, and CBCT has the advantage of being applicable in clinical settings.

In the future, the scope of studies on the issue discussed here is to be expanded, as knowledge on the parameters of reparative dentin identified with radiological techniques is still insufficient.

Ethics approval and consent to participate

The study was approved by the Local Ethics Committee of the Pomeranian Medical University in Szczecin, Poland (approval No.: KB/2020/47/NK). All patients provided written informed consent to participate 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

Not applicable.

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