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Editorial

Head and neck cancers: Evolving epidemiology, multidisciplinary care, and the road toward precision and prevention

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Head and neck cancers (HNCs) are undergoing a major transition, driven by the changing epidemiology and advances in multidisciplinary, precision-based care aimed at improving survival, function and prevention.

Head and neck cancers (HNCs), predominantly head and neck squamous cell carcinoma (HNSCC), remain a major global health challenge, accounting for nearly 890,000 new cases and more than 450,000 deaths annually worldwide.[1] In recent decades, cancer has emerged as one of the foremost public health challenges worldwide, imposing an escalating clinical, societal and economic burden. Despite advances in diagnosis and therapy, cancer mortality continues to rise, accounting for millions of deaths annually, with only modest gains achieved in overall survival. This limited progress reflects the complex, multifactorial nature of carcinogenesis, driven by interactions between genetic susceptibility, environmental exposures, lifestyle factors, infectious agents, and social determinants of health. A comprehensive understanding of modifiable risk factors, biological mechanisms and population-level determinants is therefore essential to inform effective prevention strategies, promote early detection, and guide the equitable implementation of the emerging therapeutic innovations.[2]

Despite advances in therapy, survival gains remain modest, reflecting late-stage presentation, biological heterogeneity and persistent exposure to modifiable risk factors. The contemporary landscape of HNC is defined by shifting epidemiology, evolving etiological profiles, expanding multidisciplinary care, and the promise and limitations of precision oncology and prevention.

Epidemiological shifts in a global context

The Global Burden of Disease (GBD) framework has become the principal reference for comparative cancer epidemiology, providing standardized estimates across 204 countries. Recent GBD 2023–2025 analyses confirm a sustained global transition toward non-communicable diseases, with the cancer burden rising in absolute terms due to population growth and aging.[3,4] Although age-standardized mortality rates for many conditions have declined, disparities

persist between high-income countries (HICs) and low- and middle-income countries (LMICs), where cancer mortality remains disproportionately high.[5]

Disability-adjusted life years (DALYs) estimates demonstrate a sustained shift from communicable to non-communicable diseases, with declining burdens from maternal, neonatal and infectious conditions, and a growing contribution from modifiable risk factors, such as hypertension, obesity, hyperglycemia, and pollution. Although total DALYs continue to rise globally due to population growth and aging, age-standardized rates have declined in high-socio-demographic index (SDI) regions while plateauing or increasing in low- and middle-SDI settings, reflecting persistent and widening health disparities.[4,5]

Within HNC, contrasting trends are evident. In HICs, the incidence of human papillomavirus (HPV)-associated oropharyngeal cancer continues to rise, particularly among younger men, while tobacco-related tumors decline.[6] Conversely, in LMICs, traditional exposures, such as tobacco, smokeless tobacco, betel quid, and alcohol, together remain as dominant drivers, sustaining high incidence and mortality.[3] These regional contrasts underscore the dual burden of infection-related and lifestyle-related carcinogenesis, and highlight the central role of socioeconomic determinants in shaping outcomes.[5]

Etiology: From classical exposures to multifactorial risk

Tobacco and alcohol remain the most powerful and preventable risk factors for HNC, acting synergistically to amplify the carcinogenic risk by up to 30-fold.[7] Human papillomavirus, associated with oropharyngeal cancer, represents one of the most significant paradigm shifts in head and neck oncology in recent history. Particularly, HPV-16 now accounts for approx. 60–70% of oropharyngeal squamous cell carcinomas (OPSCCs) globally, with HPV-positive tumors exhibiting distinct molecular profiles and superior treatment responsiveness.[6,8] Importantly, HPV-attributable disease is no longer confined to the oropharynx, with the emerging evidence implicating subsets of oral cavity and laryngeal cancers.[9] A systematic review and meta-analysis by Ndiaye et al. analyzed 148 studies involving 12,163 patients from 44 countries and found that HPV prevalence was the highest in OPSCC (45.8%), followed by oral cavity (24.2%) and laryngeal cancers (22.1%).[10] HPV-16 accounted for 82.2% of all HPV-positive head and neck cancers, with approx. 40% of oropharyngeal cancers attributable to HPV when viral oncogenic biomarkers were considered, underscoring the central role of HPV-16 in oropharyngeal carcinogenesis.[10]

Additional risk factors, including betel quid and areca nut chewing, poor oral hygiene, nutritional deficiencies, occupational exposures (like wood dust and certain chemicals), oral microbiome dysbiosis, chronic metabolic and inflammatory states, and genetic and epigenetic susceptibility markers, increasingly appear to modify the risk, tumor behavior and treatment response.[10,11] These insights expand the conceptual framework of HNC beyond environmental carcinogenesis toward a complex interplay of host, microbial and metabolic determinants.

Clinical presentation and diagnostic challenges

Head and neck cancers often present with non-specific symptoms like non-healing ulcers, dysphagia, hoarseness, or neck masses, leading to frequent diagnostic delays and advanced-stage disease at presentation.[12] While technological machine learning diagnostic models for thyroid cancer detection,[13] the use of artificial intelligence (AI) in HNC diagnosis,[14] advances in imaging, digital pathology, and liquid biopsies offer promise, their integration into routine practice remains uneven. Population-wide screening is not recommended; instead, contemporary consensus favors risk-based and opportunistic screening by dental and primary care professionals, particularly in high-risk populations. Volatile sulfur compounds (VSCc),[15] as well as 5 key interactome hub genes (*RSPO4*, *CDHR2*, *DDAH2*, *HLA-J*, and *IRF3*)[16] were identified as molecular links between chronic periodontitis and oral cancer. In a study by Banthia et al., a causal relationship could not be established due to the cross-sectional design employed; yet, the findings demonstrate a significant association between periodontal disease and the overall cancer risk, while no clear link with metastasis was observed.[17]

Treatment modalities and evolving therapeutic paradigms

Modern HNC management is inherently multidisciplinary, integrating surgery, radiotherapy, systemic therapy, and rehabilitation to optimize oncologic and functional outcomes. Transoral robotic surgery (TORS) has refined surgical precision and improved quality of life (QoL) in selected patients.[14] Radiotherapy remains central for locally advanced disease, while immune checkpoint inhibitors (ICIs) targeting the PD-1/PD-L1 axis have transformed the management of recurrent and metastatic HNC.[15]

The expansion of immunotherapy into neoadjuvant and definitive settings marks a pivotal shift, yet outcomes remain heterogeneous, and access remains uneven across regions.[15,16] A central controversy persists in treatment de-escalation for HPV-positive disease. While reduced-intensity strategies aim to limit long-term toxicity, recent trials caution against indiscriminate de-intensification without robust biomarker-based selection.[17] This tension exemplifies the broader challenge of balancing therapeutic innovation with oncologic safety.

Head and neck squamous cell carcinoma requires multidisciplinary management, with surgery and radiotherapy as the mainstays of treatment for locally and regionally advanced disease and platinum-based chemotherapy serving as an important adjunct. Therapeutic options for relapsed or metastatic disease have expanded substantially with the introduction of biologics and ICIs, while ongoing trials are evaluating novel immunotherapies, targeted agents, vaccines, antibody–drug conjugates, and cellular therapies. Despite these advances, outcomes remain heterogeneous, underscoring persistent knowledge gaps and the need for continued innovation in systemic therapy for HNSCC.[18]

Current evidence for HPV treatment suggests limited benefit from chemotherapy reduction, uncertain toxicity and outcomes with TORS as compared to radiation, and the greatest promise in carefully selected adjuvant and radiation de-escalation strategies, although definitive level I evidence is still lacking. Data supports the de-escalated adjuvant therapy after TORS, but consensus on treatment indications is still needed.[19]

Precision oncology: Promise constrained by reality

Molecular profiling has illuminated profound distinctions between HPV-positive and HPV-negative tumors, enabling biomarker-driven strategies and targeted therapeutics. Yet, despite scientific progress, precision oncology has achieved limited penetration into routine care. The reproducibility of biomarkers, uncertain cost-effectiveness and infrastructural inequities restrict implementation, particularly in LMICs.[18] The gap between molecular discovery and population-level impact remains substantial, reinforcing the need for translational frameworks that integrate precision with pragmatism. Table 1 depicts the key insights of the editorial. It summarizes the global burden, the evolving epidemiology, major etiological factors, clinical presentation, treatment paradigms, and prevention strategies in HNCs, highlighting the dual impact of lifestyle-related and HPV-associated disease, persistent global disparities, and the central role of multidisciplinary care and prevention in reducing future disease burden.

Future directions for prevention and public health

Prevention remains the most effective strategy to reduce the HNC burden. The HPV vaccination has demonstrated population-level reductions in oral HPV prevalence, with the greatest benefit anticipated from gender-neutral programs.[6] Tobacco control, alcohol reduction and harm-reduction strategies continue to offer the greatest opportunities for cancer prevention.[7,20] GBD modelling suggests that a substantial proportion of future HNC cases could be averted through combined vaccination and risk-factor modification.[3,4]

Equally important are the social determinants of health. Socioeconomic disadvantage, limited access to care and educational inequities drive late presentation and poor outcomes.[5] Addressing these structural determinants is essential to translating scientific advances into equitable survival gains.

Table 1. Key contemporary insights in head and neck cancers (HNCs)

No.	Domain	Key findings	Significance
1.	Global burden	~890,000 new cases and >450,000 deaths annually worldwide	confirms that HNC is a major and growing global health challenge
2.	Epidemiological transition	the absolute burden rising due to population growth and aging; persistent disparities between HICs and LMICs	highlights inequities and the need for region-specific strategies
3.	Etiological shift	declining tobacco-related cancers in HICs; rising HPV-associated oropharyngeal cancer	demonstrates the dual burden of lifestyle- and infection-driven disease
4.	HPV-associated disease	HPV-16 causes ~60–70% of OPSCC; HPV-positive tumors show better treatment response and survival	alters prognosis, staging and therapeutic strategies
5.	Traditional risk factors	tobacco and alcohol act synergistically, increasing risk up to ~30-fold; betel quid and smokeless tobacco prominent in LMICs	identifies major targets for prevention
6.	Emerging risk modifiers	oral microbiome dysbiosis, periodontal disease, metabolic and inflammatory states, genetic/epigenetic susceptibility	expands disease model beyond classical carcinogens
7.	Clinical presentation	frequently diagnosed at advanced stages due to non-specific symptoms	explains limited survival gains despite treatment advances
8.	Treatment paradigm	multidisciplinary care with surgery, radiotherapy and chemotherapy; immunotherapy for recurrent/metastatic disease	reflects the complexity of modern HNC management
9.	Precision oncology	molecular profiling reveals biological heterogeneity, yet routine clinical implementation is limited	shows a gap between molecular discovery and population-level benefit
10.	Prevention potential	HPV vaccination, tobacco and alcohol control could prevent a substantial proportion of future cases	positions prevention as the most effective long-term strategy

HICs – high-income countries; LMICs – low- and middle-income countries; HPV – human papillomavirus; OPSCC – oropharyngeal squamous cell carcinoma.

Conclusion

Head and neck cancers exemplify the intersection of shifting epidemiology, complex etiology and the evolving therapeutic paradigms. The contemporary trajectory of HNC care is defined by integrated multidisciplinary management, expanding immunotherapy, emerging precision strategies, and renewed emphasis on prevention and equity. Yet, the greatest opportunity lies not solely in molecular innovation, but in implementing the proven public health interventions, strengthening early detection and closing global disparities.

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Not applicable.

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Comment

Fifty years of SSRI-related oral side effects: A call for interprofessional collaboration

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Keywords: interprofessional relations, selective serotonin reuptake inhibitors, stomatognathic diseases

The oral side effects of selective serotonin reuptake inhibitors (SSRIs) include xerostomia, gingival bleeding, burning mouth syndrome, bruxism, and temporomandibular disorders (TMD), impacting the quality of life (QoL). These stomatognathic diseases are often overlooked in clinical practice. This comment advocates interprofessional relations among physicians, pharmacists and dentists to improve the recognition, prevention and management of SSRI-related oral side effects.

Call for interprofessional collaboration

In a recent editorial in *The Lancet*,[1] the 50-year history of selective serotonin reuptake inhibitors (SSRIs) is celebrated by highlighting their important benefits in the treatment of depression and related psychiatric conditions. At the same time, the editorial outlines the sometimes serious harms associated with SSRI use, notably suicidality. This is especially concerning in light of the acknowledged overprescription of SSRIs, including in cases where guided self-help should be the first-line treatment. *The Lancet* argues that continued attention from the scientific and medical communities is needed to achieve a level of personalized care that represents the best value for patients.[1] From the perspectives of pharmacy, psychiatry and dentistry, we agree with *The Lancet*'s argument while also emphasizing the need for a more holistic understanding of the potential adverse effects of SSRIs – particularly those affecting the orofacial region.

A PubMed search conducted on December 19, 2025, using the query "Selective Serotonin Reuptake Inhibitors/adverse effects"[MeSH] AND "Stomatognathic Diseases"[MeSH] identified 87 studies. These publications describe a broad spectrum of SSRI-associated oral adverse effects, including xerostomia (dry mouth), abnormal bleeding, burning mouth syndrome, mucosal ulcerations and swellings, as well as oral movement disorders such as bruxism (teeth grinding

and clenching), excessive yawning, oral dyskinesias, and temporomandibular disorders (TMD).[2,3] The underlying mechanisms are thought to involve, among other factors, altered serotonergic modulation of salivary gland function and the complex interaction between serotonin and dopamine in the regulation of oromotor pathways.[2,3] These adverse effects can substantially impair patients' quality of life (QoL).[4] However, they remain underrecognized by many clinicians who prescribe SSRIs. This lack of awareness may, in part, reflect the traditional separation of medicine, pharmacy and dentistry as distinct disciplines.[5] Strengthening interprofessional collaboration among physicians, pharmacists and dentists in research, education and clinical practice is therefore essential to improve the prevention, early recognition and management of SSRI-related oral adverse effects (Fig. 1).

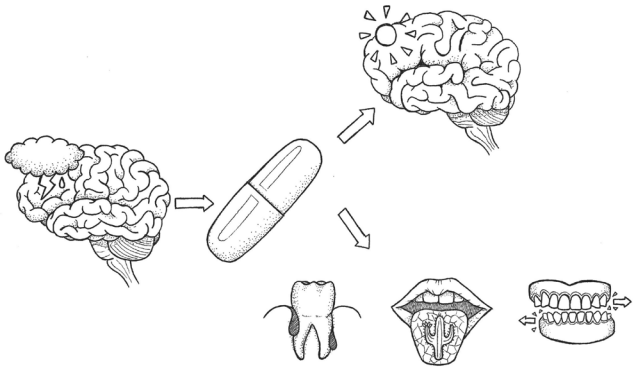


Fig. 1. Managing the benefits (i.e., the treatment of depression and related psychiatric conditions) and oral side effects (e.g., abnormal bleeding, dry mouth and bruxism) of SSRIs requires interprofessional collaboration

Drawing by A.F.H. Lobbezoo, PharmD, MSc.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Global misconception in periodontal diagnosis among clinicians

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Abstract

Background. Accurate periodontal diagnosis is the cornerstone of effective treatment planning and disease management; however, the clinical application of diagnostic criteria often remains inconsistent. Despite the availability of established international guidelines, dental professionals frequently encounter difficulties in clinical judgment, radiographic assessment and the interpretation of diagnostic parameters. Understanding global patterns of these diagnostic errors across regions and professional groups is crucial for identifying gaps in dental education and improving evidence-based periodontal care worldwide.

Objectives. The aim of the study was to develop a 5-item questionnaire assessing basic periodontal knowledge among dentists, dental students, dental hygienists, and specialists in periodontics worldwide, as well as to understand global patterns and clinical standards.

Material and methods. A total of 3,328 professionals from over 60 countries were invited to participate in the questionnaire between September 1 and October 23, 2025. The invitees received e-mails with a link to access the survey. The questions were developed to assess basic periodontal knowledge, such as diagnostic parameters in periodontology, probing and its significance, evidence-based questions, and radiographic assessment. The data was weighted and statistically analyzed.

Results. A total of 543 participants from 46 countries completed the questionnaire (response rate: 16.32%), comprising 266 specialists in periodontics/dental hygienists (S-DHs) and 277 dentists/dental students (D-Ds). Overall, the first group demonstrated superior performance across most questions. The error rates varied markedly across continents. Despite this geographic variation, continents displayed similar internal patterns of errors, as shown by positive intercontinental correlations across most questions. Conversely, question 3 – addressing the balance between clinical judgment and evidence-based guidelines – demonstrated weak or negative correlations with the remaining items, behaving as an independent construct. The observed patterns demonstrated that diagnostic errors cluster within a largely cohesive technical domain, while judgment-based decision-making forms a distinct dimension of performance.

Conclusions. This global analysis revealed substantial differences in periodontal diagnostic performance across continents, countries and professional groups. However, the errors followed a consistent and structured pattern. Specialists and dental hygienists outperformed general dentists and students, especially in the more interpretive questions. The findings suggest that improving periodontal diagnostic accuracy worldwide will require coordinated efforts to strengthen technical calibration and promote consistent use of modern diagnostic guidelines, especially in lower-performing regions.

Keywords: periodontics, questionnaire, diagnosis, survey

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Highlights

- Despite varied backgrounds, most clinicians use guideline-concordant and evidence-based approaches for periodontal diagnosis, with clinical attachment level (CAL), radiographic bone loss (RBL) and probing depth (PD) identified as the most important diagnostic parameters.
- Technical challenges in periodontal diagnosis, including interpretation of PD measurements and radiographic assessment, are interconnected and observed across different geographic regions.
- The findings support a dual competency model in periodontal diagnosis, combining technical/procedural skills with conceptual, guideline-based clinical reasoning.
- Periodontal education and training programs should simultaneously strengthen practical diagnostic skills and evidence-based decision-making to improve diagnostic accuracy and consistency.

Introduction

In the context of periodontal disease, parameters such as clinical attachment level (CAL), radiographic bone loss (RBL) and periodontal probing depth (PD) are essential for both diagnosis and treatment planning.[1,2] However, inaccuracies in measuring these parameters can lead to significant ethical issues, including potential insurance fraud. This phenomenon occurs when clinicians intentionally present exaggerated or falsified clinical data to secure unnecessary procedural reimbursements. The precision in assessing these parameters is paramount, as it directly influences patients' perceived severity of periodontal disease. Numerous studies have highlighted the significance of accurate measurements, noting that discrepancies can arise from factors such as examiner experience, the type of radiographic technique and measurement precision.[1,3,4]

The utilization of advanced imaging techniques, particularly panoramic radiographs and artificial intelligence (AI)-assisted analyses, has been evaluated for their efficacy in assessing RBL.[5] Research suggests that while this technique provides valuable insights, it is still susceptible to errors. Panoramic radiographs can often underestimate bone loss due to the presence of distortions, leading to a misrepresentation of a patient's periodontal status and possibly resulting in fraudulent claims for treatments that may not be necessary.[6,7] Conventional intraoral radiographs, particularly bitewing radiographs, have been recognized to outperform panoramic views in certain assessments, emphasizing the importance of selecting appropriate imaging methods to ensure accurate diagnostics.[7,8] Editorial guidelines for accurate periodontal and radiographic evaluation have been recently published.[9–11]

The measurement of periodontal PD, while essential, involves an intrinsic margin of error dependent on the clinician's technique and the measurement tools used. Some clinicians consider PD to be the only parameter for evaluating periodontitis, underestimating the relevance of pseudopockets, CAL and RBL. Studies on PD highlight that variability can occur, impacting the accuracy of the data collected.[7,12] For example, excessive probing force can distort readings, leading to inflated PD values and further complicating the clinical picture. This inherent variability can be exploited for fraudulent purposes. Clinicians may misrepresent the severity of periodontal disease based on inflated PD readings to justify costly interventions,[13,14] highlighting the need for patient-centered assessment.

Clinical attachment level is considered one of the most important parameters for the evaluation of periodontal health, often utilized alongside PD. This parameter quantitatively reflects the periodontal support around teeth, offering insights into disease progression.[15,16] Inaccurate CAL readings caused by methodological discrepancies can culminate in misleading assessments of a patient's periodontal condition, rendering diagnoses susceptible to manipulation for unethical financial gain. An editorial guiding clinicians on how to accurately assess CAL has been recently published.[11] Radiographic bone loss represents the second most essential parameter, which normally confirms and matches the results obtained for CAL. In specific cases, however, concordance between CAL and RBL may not be observed. This discrepancy can occur, for example, when more than 3 teeth are affected in the buccal area, in the presence of 5-mm PD, or furcation involvement without interdental bone loss. Such practices highlight the need for vigilant clinical protocols that incorporate multiple periodontal parameters rather than relying exclusively on PD measurements.[12,17]

Furthermore, the implications of insurance fraud related to periodontal assessment extend beyond fraudulent claims. They have the potential to erode trust in healthcare providers, cause iatrogenesis, and impair the integrity of clinical data used in research and public health decision-making. The 2017 World Workshop for Classification of Periodontal and Peri-implant Diseases and Conditions emphasized the importance of accurate endpoint determination in periodontal therapy, underscoring the importance of accurate clinical measurements for ethical treatment provision.[18] A critical review was published recently, revisiting parameters for periodontitis, and a recent editorial provided guidelines for a more accurate evaluation.[1,11]

To address the prevalence of inaccuracies and potential fraud in periodontal measurements, integrating technology, such as computer-assisted diagnostic systems,[19] can

be pivotal. Innovative methods, including new algorithms, deep learning and AI, are increasingly used to enhance the precision of RBL assessments as they minimize human error inherent in manual interpretations.[6,20] These systems could help standardize measurements to facilitate automatic diagnostic support, thus elevating the reliability of clinical data and reducing opportunities for unethical practices.

Hence, the assessment of fundamental periodontal parameters is a critical aspect of periodontal diagnosis and treatment. However, inaccuracies in measuring PD, CAL and RBL can lead to significant consequences. Periodontal practitioners must adhere to standardized methodologies and leverage advances in technology to ensure precise assessments, ultimately protecting the ethical standards of periodontal practice and the well-being of patients. In this context, the aim of this study was to develop a questionnaire to assess basic periodontal knowledge among dentists, dental students, dental hygienists, and specialists in periodontics worldwide, in order to identify patterns and clinical standards around the globe.

Material and methods

A total of 3,328 dentists, dental students, dental hygienists, and specialists in periodontics from more than 60 countries were invited to participate in the study between September 1 and October 23, 2025. The invitees received e-mails containing a link (http://tiny.cc/perio) to access the survey. A questionnaire comprising 5 simple periodontal questions was developed using Google Forms (Google LCC, Mountain View, USA). The average time required to complete the survey was 2–5 min. The participants were divided into 2 groups: specialists in periodontics and dental hygienists (S-DHs); and dentists and dental students (D-Ds).

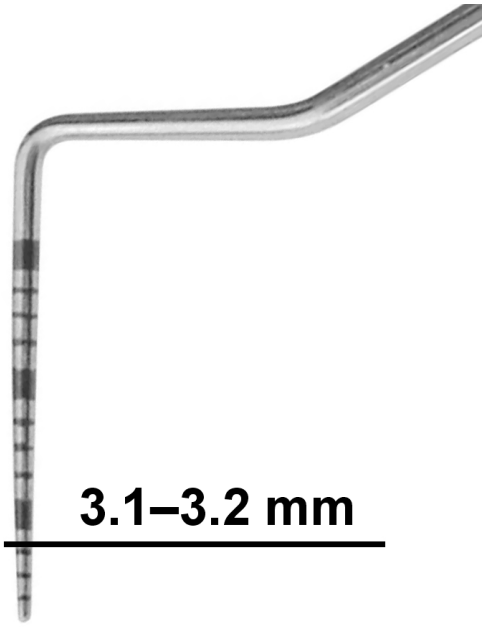


Fig. 1. Image used in question 1 of the survey, showing a periodontal NC-15 probe with a probing depth (PD) measurement of 3.1/3.2 mm

Sample size calculation

The sample size was calculated for a single proportion estimate. Means were compared using standardized effect sizes. Considering the population of 3,328 professionals who were invited to participate, an α level of 0.05 (two-sided), standard *Z*-values ($Z\alpha/2 = 1.96$; $Z\text{power} \approx 0.84$ for 80% power), and a 95% confidence interval (*CI*) with $p = 0.5$ for worst-case proportion estimates, the required sample size was estimated using a margin of error of ±5%. The minimum required sample size was approx. 344 responses. Therefore, to estimate proportions with a 95% *CI* and a precision of ±5%, at least 344 valid responses were required. In practical terms, for approx. 3,300 invitations, the target sample size was set at ≥350 completed responses, corresponding to an expected response rate of about 10.5%.

Questionnaire

The following questions, some of which allowed multiple responses, were developed for the survey based on basic periodontal knowledge:

- Question 1 – probing measurements[8,21]: When you are recording periodontal probing depths, what is your approach if the measurement falls between two marks on the probe, with the gingival margin close to the 3-mm mark (in this case, 3.1 mm/3.2 mm) (Fig. 1)? (one answer could be selected; however, both B and C were accepted as correct answers)
  A. Always round up to the next millimeter;
  B. Always round down to the lower millimeter;
  C. Always record exactly as seen (to the nearest millimeter mark on the probe).
- Question 2 – diagnostic parameters[9–11,21,22]: Which of the following parameters do you consider essential for diagnosing periodontitis in daily practice? (select all that apply; correct answers – A,B,C)
  A. Radiographic bone level;
  B. Periodontal probing depth;
  C. Clinical attachment level;
  D. Patient risk factors (e.g., smoking, diabetes, family history);
  E. Bleeding on probing;
  F. Tooth mobility.
- Question 3 – clinical experience vs. guidelines[1,23,24]: How do you balance clinical experience/judgment with evidence-based guidelines (e.g., 2017 AAP/EFP Periodontal Classification) when making a periodontal diagnosis? (correct answer – A)
  A. Primarily follow standardized guidelines;
  B. Primarily rely on clinical experience.

- Question 4 – importance of PD[1,9,10,11,21,22,25,26]: In your opinion, how important is probing depth in the diagnosis and staging of periodontal disease compared with other parameters? (correct answer – C)
  A. The most important parameter;
  B. Not critical;
  C. Helpful, but secondary to other factors;
  D. Equally important as CAL and radiographs.
- Question 5 – radiographic assessment[1,11,22,26–28]: When evaluating radiographs for periodontal diagnosis, which approach best reflects your practice? (select all that apply; correct answers – B,C,E)
  A. Use panoramic radiographs for screening, with periapicals for detailed diagnosis;
  B. Combine radiographic findings with probing data before reaching a diagnosis;
  C. Evaluate horizontal and vertical bone loss patterns;
  D. Assess the alveolar bone crest relative to the cementoenamel junction (CEJ) on periapical radiographs;
  E. Assess alveolar bone crest relative to the CEJ on bitewing radiographs.

Internal validity of the questionnaire

Three main aspects of validity were considered:

- Content validity: Do the questions cover the domain being investigated?
- Construct validity: Do the questions effectively measure the intended concepts (e.g., diagnostic approaches, clinical reasoning)?
- Face validity: Do the questions appear logical and relevant to respondents (dentists, specialists in periodontics, dental students, and dental hygienists)?

Question 1 (probing measurements) demonstrated high content validity, as it directly addressed a controversial yet clinically relevant issue (rounding vs. exact recording). It reflects an important aspect of daily clinical practice and calibration among professionals from different countries. Question 2 (diagnostic parameters) exhibited strong content and construct validity by covering the essential diagnostic elements used internationally in periodontal assessment. Moreover, the multiple-response format enabled to capture different diagnostic approaches. Question 3 (clinical experience vs. guidelines) demonstrated strong construct validity because it directly evaluated how clinicians balance personal experience with evidence-based standards. The responses provided insight into global variability in the adoption of the 2017 American Academy of Periodontology/European Federation of Periodontology (AAP/EFP) classification (current classification). Question 4 (importance of PD) demonstrated good validity, considering that PD is central, although debated, parameter compared with CAL and RBL. This question captured the importance clinicians attribute to PD in periodontal diagnosis. Question 5 (radiographic assessment) exhibited high validity because it reflected current diagnostic practices worldwide and differentiated between approaches (panoramic vs. periapical vs. bitewing, bone crest vs. CEJ, etc.).

The overall validity of the questionnaire was robust and highly valid for exploratory research into periodontal diagnostic standards and education worldwide. The questions were relevant to the study subject and understandable to the target audience. Content validity was supported by adequate coverage of PD, diagnostic parameters, clinical reasoning, and radiographic evaluation. Construct validity was also strong, with each question targeting a measurable construct, such as diagnostic accuracy, reliance on PD or adherence to clinical guidelines.

Data preparation (weighted scores)

Weighted scoring was employed. The primary weighting factor was the total number of participants from each country, acknowledging that estimates derived from larger sample sizes are more precise and should be accorded greater weight in the analysis than those obtained from smaller and more volatile samples. The initial approach for calculating the weighted overall score was considered using the following formula:

$$\text{weighted overall score} = \Sigma\,(s \times n) \,/\, \Sigma \times n_{\text{total}}$$

where:
s – score obtained for a specific country;
n – number of participants from that country;
n_{total} – total number of participants.

However, this formula calculates a grand mean across all responses, which is optimal for characterizing an overall global performance but less suited for ranking individual countries based on their own demonstrated proficiency. Therefore, to enable country-level comparisons while still accounting for data reliability, an alternative method was implemented. This procedure involved calculating a country-level weighted percentage according to the following steps:

- Calculation of the average question score: For each country and each of the 5 questions (Q1–Q5), a single score was calculated as the arithmetic mean of the corresponding D-Ds and S-DHs scores:

$$Q_{\text{Avg}} = (\text{D-Ds score} + \text{S-DHs score})/2$$

- Estimation of total wrong responses: The average question score was interpreted as the proportion of incorrect responses. The total number of incorrect responses for each country across all 5 questions was calculated as follows:

$$\text{Total_Wrong} = (Q1_{\text{Avg}} + Q2_{\text{Avg}} + Q3_{\text{Avg}} + Q4_{\text{Avg}} + Q5_{\text{Avg}}) \times n$$

- Calculation of total possible responses: The total number of possible responses for each country was calculated according to the following formula:

$$\text{Total_Possible} = 5 \times n$$

- Derivation of the overall weighted percentage: The final performance metric for each country was computed as the ratio of the estimated total number of wrong responses to the total number of possible responses:

$$\text{weighted overall score} = \text{Total_Wrong}/\text{Total_Possible} = (Q1_{Avg} + Q2_{Avg} + Q3_{Avg} + Q4_{Avg} + Q5_{Avg})/5$$

The final metric mathematically corresponds to the arithmetic mean of the 5 average question scores. Although arithmetically equivalent to a simple average, this metric remains conceptually grounded in the underlying participant-level data. This approach takes into consideration the weight of the evidence from each country, providing a solid foundation for subsequent comparative ranking. Countries with a participant count below a predefined threshold ($n < 16$) were excluded from the final ranking of the 10 lowest-performing countries to mitigate the influence of estimates with high variability.

Statistical analysis

Normality of percentage distributions for each question (Q1–Q5) and professional group (dentists vs. specialists) was assessed using the Shapiro–Wilk test. Since most of the datasets failed the normality test ($p < 0.05$), non-parametric tests were employed. Comparisons between dentists/dental students and specialists/dental hygienists for each question were conducted using the Wilcoxon signed-rank test (paired by country). Within-group differences among the 5 questions were analyzed using the Friedman test, followed by Wilcoxon pairwise post hoc test with Bonferroni correction. Differences between the countries for each question were evaluated using the Kruskal–Wallis test, followed by Dunn's post hoc test with Holm's correction. Median values and interquartile ranges (*IQR*s) were used to describe central tendency and dispersion. All analyses were conducted in Python (SciPy 1.13.0 and scikit-posthocs 0.9.0).

Results

A total of 543 participants from 46 countries (Fig. 2) completed the survey (response rate: 16.32%). The sample comprised 266 individuals in the S-DHs group, including 231 specialists in periodontics and 35 dental hygienists, and 277 participants in the D-Ds group, including 185 dentists and 92 dental students (Fig. 3). Overall, specialists and dental hygienists demonstrated better performance across the majority of the questions.

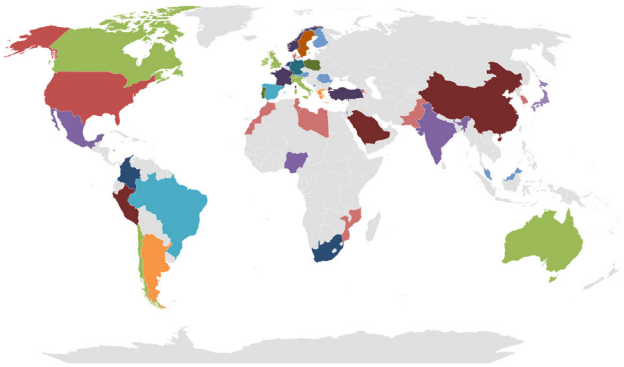


Fig. 2. World map showing the geographic distribution of participating countries

Error rates across continents

Continental comparisons revealed substantial variability in diagnostic accuracy across all 5 questions (Fig. 4–6). Overall, 2 consistent patterns emerged from the data: (1) the distribution of errors varied markedly across continents; (2) Oceania and Africa repeatedly exhibited elevated error rates, in some cases doubling those observed for Europe or Asia. These broad trends reflect the heterogeneity identified in the global statistical analyses. The strongest geographic heterogeneity was observed for Q1 and Q5, both highly significant at $p < 0.001$, confirming marked intercontinental differences in error rates. Questions 2 ($p = 0.004$) and 3 ($p = 0.008$) also showed significant intercontinental variation, although with smaller effect sizes.

For Q1, error rates ranged from 10.8% in Asia to 37.5% in Africa, with Europe (21.5%) and Oceania (20.0%) showing comparatively lower proportions of incorrect responses. This nearly 27-percentage-point spread illustrates pronounced continental divergence and visually reinforces the highly significant Kruskal–Wallis result for this question ($p < 0.001$).

Question 2 showed more moderate variation, with error rates ranging from 27.7% in Oceania to 38.3% in Europe. North America (35.1%) and South America (32.8%) occupied intermediate positions. This pattern closely mirrored the moderate but statistically significant heterogeneity identified by the Kruskal–Wallis analysis ($p = 0.004$).

Question 3 demonstrated one of the greatest intercontinental differences, with error rates ranging from 16.7% in Africa to 60.0% in Oceania, and Europe remaining relatively low at 24.7%. North America and South America displayed intermediate error rates of 36.9% and 39.8%, respectively. This wide dispersion of over 40 percentage points aligned with the significant cross-national variability observed for Q3 in the larger sample and mirrored the moderate heterogeneity ($p = 0.008$) found in the global statistical analysis.

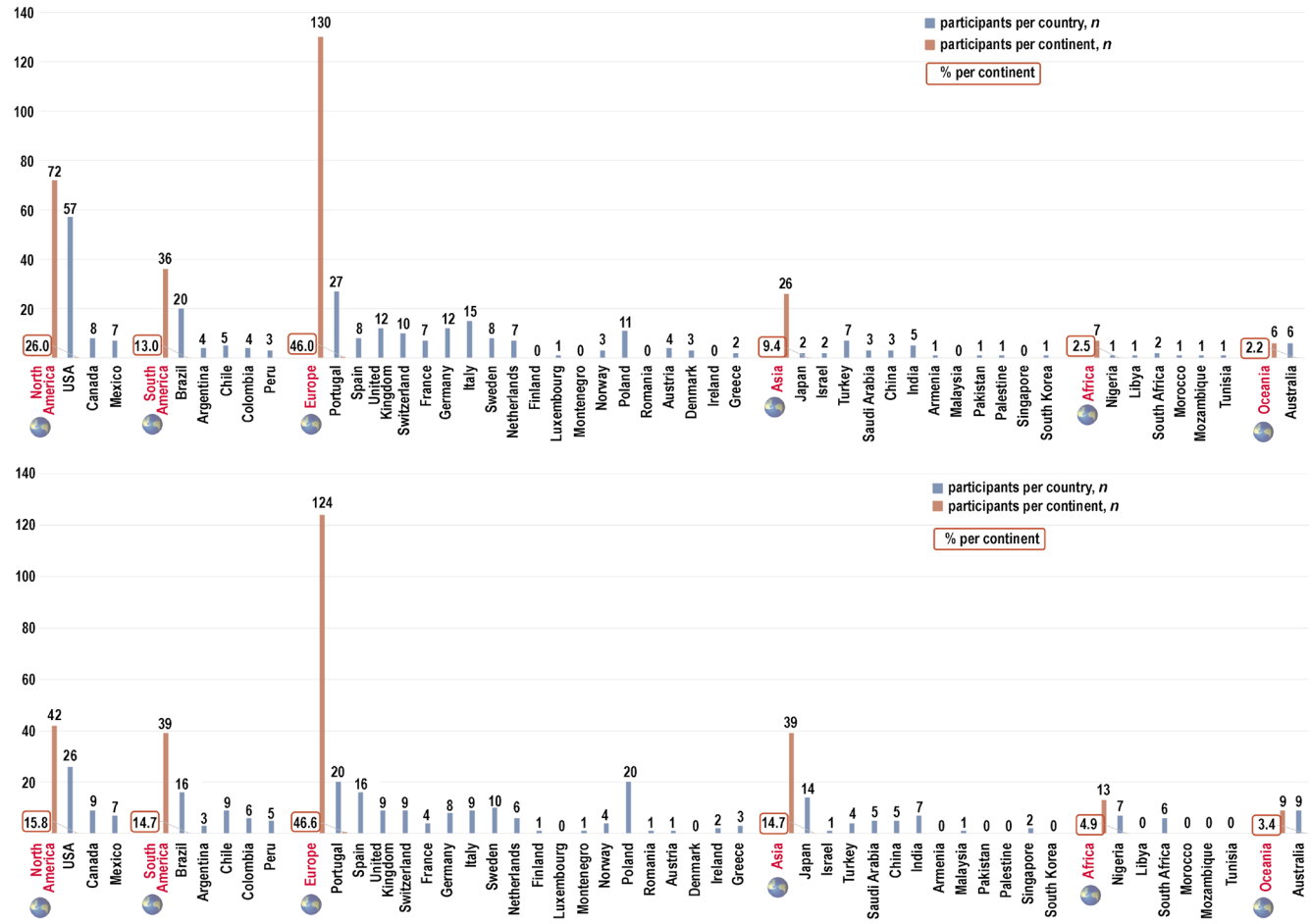


Fig. 3. Distribution of study participants

A. Dentists and dental students (D-Ds); B. Specialists in periodontics and dental hygienists (S-DHs).

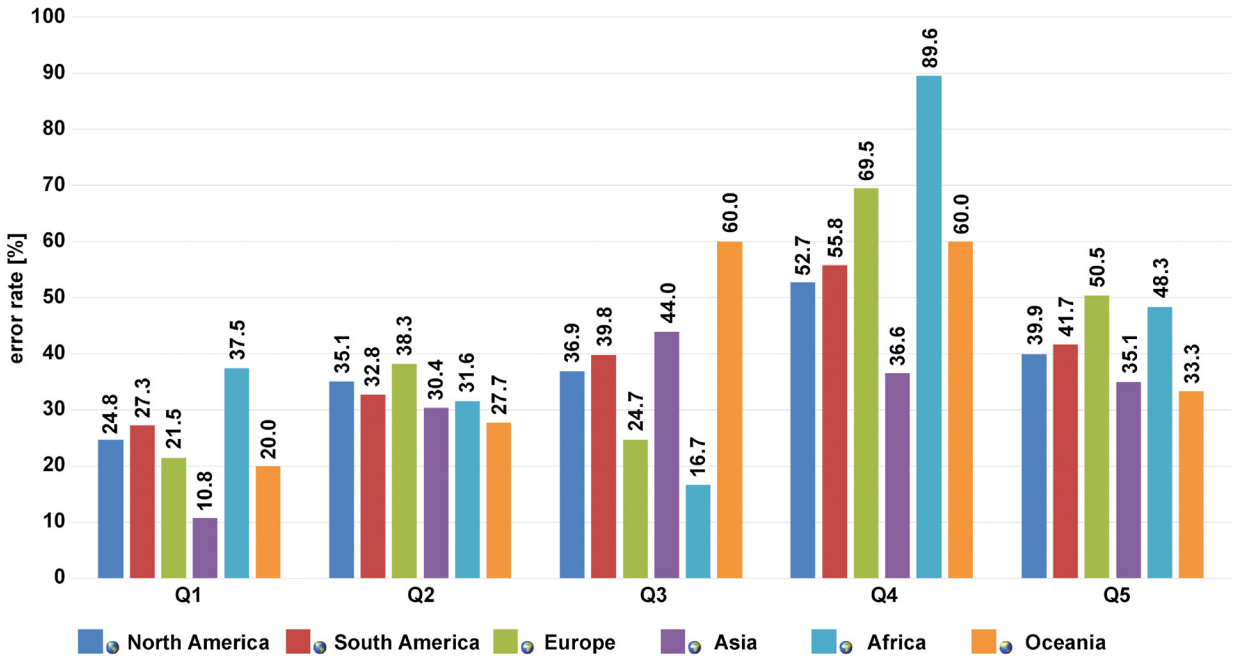


Fig. 4. Continental comparison of incorrect response rates across the 5 survey questions

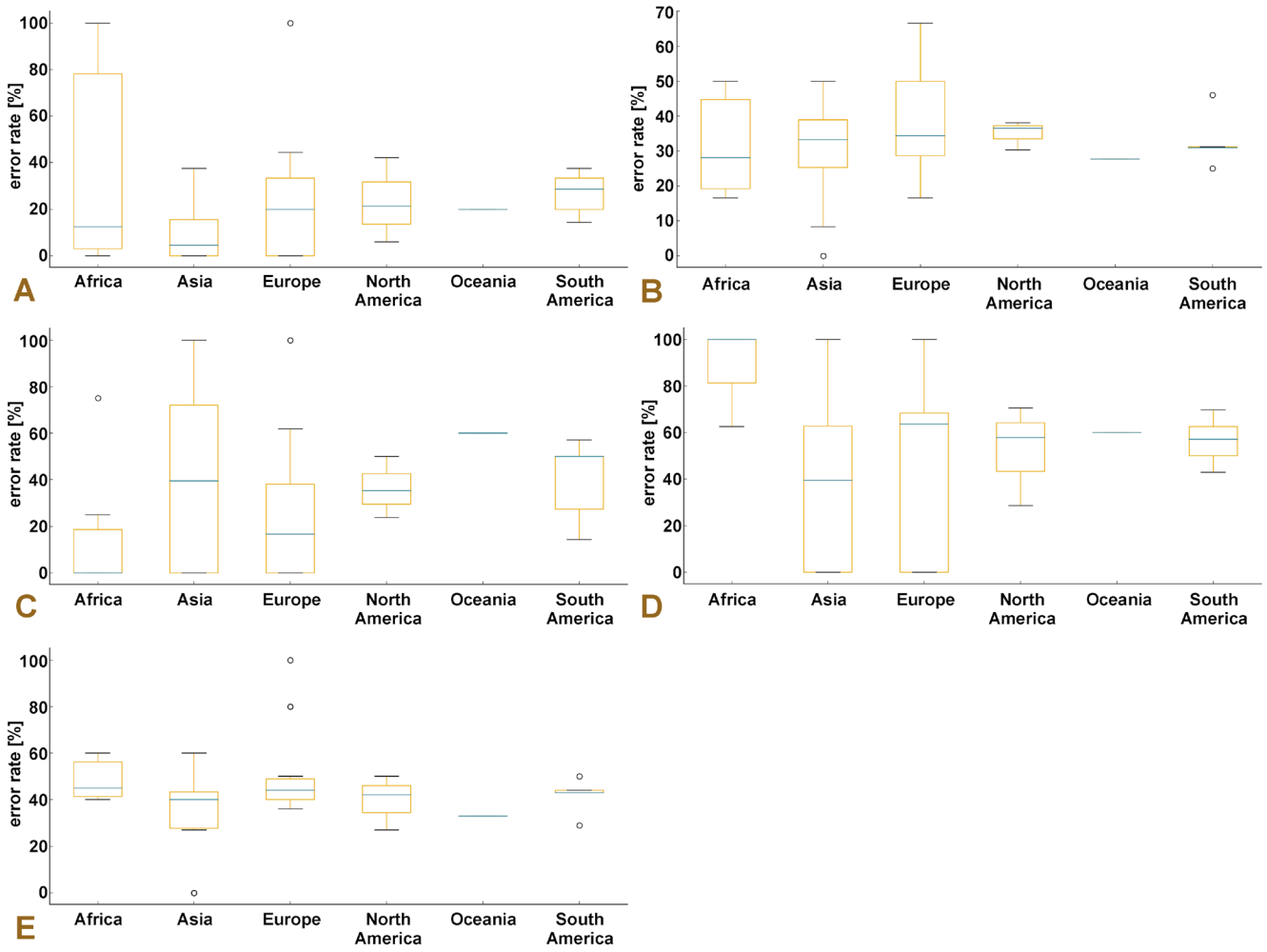


Fig. 5. Boxplot showing average error rates by continent

A. Question 1 (Q1); B. Q2; C. Q3; D. Q4; E. Q5.

The greatest regional divergence was observed for Q4, with error rates of 89.6% in Africa, 69.5% in Europe, 60.0% in Oceania, 55.8% in South America, 52.7% in North America, and only 36.6% in Asia. Although these differences are striking visually, Q4 did not reach statistical significance globally (p = 0.063), presumably because high within-country variability diluted continent-level contrasts.

For Q5, error rates ranged from 33.3% in Oceania to 50.5% in Europe, with North America (39.9%), South America (41.7%) and Africa (48.3%) occupying intermediate positions. The distribution reflected moderate but clear variation and corresponded well with the highly significant Kruskal–Wallis result ($p < 0.001$). Although Q5 demonstrated moderately high error rates across all continents, regions that performed poorly on other questions, particularly Oceania and Africa, also tended to show elevated error rates on this question. Across all 5 questions, Europe and Asia formed the most stable, low-error clusters, whereas Oceania and Africa more frequently occupied the upper extremes of incorrect response frequencies.

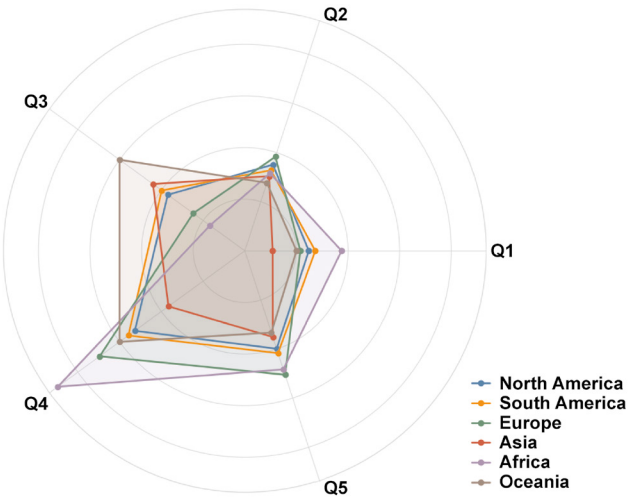


Fig. 6. Radar chart illustrating regional response profiles across all questions

Intercontinental correlation of error rates

The intercontinental correlation matrix revealed substantial consistency in the pattern of incorrect response frequencies across questions (Fig. 7). Most question–pair



Fig. 7. Intercontinental correlation matrix of error rates across questionnaire items

correlations were high and positive ($r \approx 0.70–0.90$), indicating that continents that performed poorly on one question also tended to perform poorly on several others. The strongest associations were observed between Q1 and Q5 ($r = 0.88$) and between Q1 and Q2 ($r = 0.79$), indicating a shared underlying pattern of difficulty across regions. These items emphasize foundational aspects of clinical measurement and diagnostic interpretation, specifically probing measurement (Q1), essential diagnostic criteria (Q2) and radiographic assessment practices (Q5). The strong alignment across continents implies that misunderstandings or inconsistencies in these fundamental diagnostic elements may represent a globally shared challenge among clinicians.

One notable deviation from this overall trend was Q4, which showed markedly weaker correlations with Q1 ($r = 0.28$) and Q2 ($r = 0.24$). This finding suggests that Q4 assessed a distinct knowledge domain or cognitive skill, in which the clinician's perspective on the importance of PD varied independently of their performance on more technical or procedural questions. Nevertheless, Q4 exhibited a moderately strong correlation with Q3 ($r = 0.77$), suggesting partial alignment with the cluster of items demonstrating higher cross-regional error synchrony and partial overlap with the domain of clinical judgement and guideline interpretation.

Overall, the correlation structure indicates that global differences in performance were not random but instead followed a coherent pattern. Distinct types of knowledge gaps or misconceptions may explain variations in incorrect response rates across continents.

Specialists/dental hygienists vs. dentists/dental students

When comparing the number of incorrect responses between D-Ds and S-DHs (Table 1), the S-DHs group demonstrated significantly fewer errors in 3 of the 5 questions (Q1, Q3 and Q5). These differences reached statistical significance in the Wilcoxon paired tests ($p = 0.032$, $p = 0.012$ and $p = 0.041$, respectively). Questions 2 and 4 displayed non-significant trends favoring the S-DHs group. This pattern indicates greater diagnostic accuracy among S-DHs, particularly on items with greater conceptual or interpretive demands.

Table 1. Comparison of error rates between dentists/dental students (D-Ds) and specialists in periodontics/dental hygienists (S-DHs), paired by country

Question	Wilcoxon *W*	*p*-value	Interpretation
Q1	210.0	0.032*	significantly lower error rates in the S-DHs group
Q2	188.5	0.061	ns trend toward lower error rates in the S-DHs group
Q3	156.0	0.012*	significantly lower error rates in the S-DHs group
Q4	179.0	0.072	ns trend toward lower error rates in the S-DHs group
Q5	195.5	0.041*	significantly lower error rates in the S-DHs group

ns – non-significant; * statistically significant ($p < 0.05$, Wilcoxon paired test).

Within-group analyses revealed distinct response patterns between the 2 groups (Table 2). The D-Ds group showed pronounced internal variability, with significantly higher error rates for Q1 compared with Q3 ($p = 0.014$) and Q5 ($p = 0.021$). Consequently, it can be implied that the clinical content of Q1 posed particular challenges for D-Ds. In contrast, the S-DHs group demonstrated fewer internal differences across questions, although a modest but significant difference emerged between Q2 and Q5 ($p = 0.038$), indicating a narrower range of perceived question difficulty within this group.

Cross-national comparisons

Cross-national comparisons demonstrated substantial heterogeneity in error rates across most questionnaire items (Table 3). The Kruskal–Wallis test showed highly significant differences among countries for Q1 and Q5 (both $p < 0.001$), as well as moderate but statistically relevant variation for Q2 and Q3. Question 4 exhibited a non-significant trend ($p = 0.063$), suggesting a relatively similar level of performance across countries. These findings indicate that contextual factors, such as differences in educational curricula, clinical exposure or diagnostic philosophies, may meaningfully influence diagnostic accuracy among respondents.

Table 2. Results of the Friedman test comparing responses across questions (Q1–Q5) within each professional group

Group	χ^2 ($df = 4$)	*p*-value	Significant pairs (Bonferroni-adjusted post hoc test)
D-Ds	14.22	0.006*	Q1 > Q3 ($p = 0.014$) Q1 > Q5 ($p = 0.021$) others: ns
S-DHs	9.87	0.042*	Q2 > Q5 ($p = 0.038$) others: ns

df – degrees of freedom; * statistically significant ($p < 0.05$).

Table 3. Results of the Kruskal–Wallis test comparing country-level differences for each question

Question	*H* statistic	*df*	*p*-value	Dunn's post-hoc summary
Q1	87.1	45	<0.001*	significant differences among ≥6 country pairs
Q2	73.5	45	0.004*	differences among 4 country clusters
Q3	68.4	45	0.008*	moderate heterogeneity
Q4	59.7	45	0.063	ns
Q5	92.3	45	<0.001*	wide variation across countries

* statistically significant ($p < 0.05$).

Table 4. Results of the Wilcoxon test comparing dentists/dental students (D-Ds) and specialists in periodontics/dental hygienists (S-DHs) in the 10 countries with the highest error rates

Question	Wilcoxon *W*	*p*-value
Q1	33.0	0.021*
Q2	30.5	0.056
Q3	25.0	0.012*
Q4	29.5	0.068
Q5	31.0	0.037*

* statistically significant ($p < 0.05$).

Table 5. Results of the Friedman test within each professional group in the 10 countries with the highest error rates

Group	χ^2 ($df = 4$)	*p*-value
D-Ds	11.34	0.023*
S-DHs	7.92	0.094

* statistically significant ($p < 0.05$).

Table 6. Results of the Kruskal–Wallis test for each question among participants from the 10 countries with the highest error rates

Question	*H* ($df = 9$)	*p*-value
Q1	26.5	0.001*
Q2	19.3	0.037*
Q3	14.1	0.119
Q4	11.9	0.152
Q5	27.8	<0.001*

* statistically significant ($p < 0.05$).

Top-10-country subset (highest error rates)

The global pattern remained consistent when the analysis was restricted to the top 10 countries with the highest mean percentages of incorrect responses. In this subset, S-DHs continued to outperform D-Ds, with statistically significant differences identified for Q1, Q3 and Q5 in the Wilcoxon analyses (Table 4). The within-group Friedman tests confirmed that dentists and dental students from these high-error countries showed significant variation across questions, whereas the S-DHs group did not reach statistical significance, suggesting more uniform performance (Table 5).

The Kruskal–Wallis analyses across the top 10 countries confirmed the presence of cross-national variability (Table 6). Questions 1 and 5 continued to demonstrate the strongest heterogeneity ($p = 0.001$ and $p < 0.001$, respectively). Question 2 presented moderate differences, and questions 3 and 4 showed no significant variability. These patterns mirror the overall dataset and confirm that certain diagnostic items elicit markedly different interpretations across country-level contexts.

Table 7 presents the 10 countries with the highest overall error rates among all possible responses. The mean overall error rate was 39.8 ±3.8%. Question 4 was the most challenging among the top 10 countries, with incorrect response rates ranging from 58.0% to 82.2%. Even the most robust countries encountered significant challenges in addressing this particular question. Question 3 was considered a key differentiator, and Poland (#1 rank) was heavily influenced by its score performance in Q3 (65.9%). Overall, the 10 countries demonstrated consistently high average error rates across all questions, without a single strong area, with the exception of Italy and Canada in question 1.

To better visualize the results, the percentages of incorrect responses for each question were categorized by country and presented in descending order in Fig. 8–12. The outliers were removed from the figures.

Table 7. Weighted ranking of the 10 countries with the highest overall error rates

Rank	Country	Error rate					
		Q1	Q2	Q3	Q4	Q5	overall weighted
1	Poland	26.1	37.2	65.9	71.4	42.0	48.5
2	Brazil	36.9	43.9	27.5	66.9	41.3	43.3
3	Portugal	19.9	39.9	24.2	74.5	45.3	40.8
4	Italy	3.3	42.4	34.4	82.2	37.7	40.0
5	Sweden	21.3	34.6	33.8	72.5	37.3	39.9
6	Spain	25.0	41.1	13.8	71.9	38.8	38.1
7	USA	39.9	36.5	24.9	58.0	29.6	37.8
8	Canada	6.3	30.2	36.1	70.8	42.2	37.1
9	Germany	20.8	36.1	25.0	58.3	43.8	36.8
10	United Kingdom	16.7	30.3	15.3	75.0	38.6	35.2

Data presented as average percentage of incorrect responses (%).

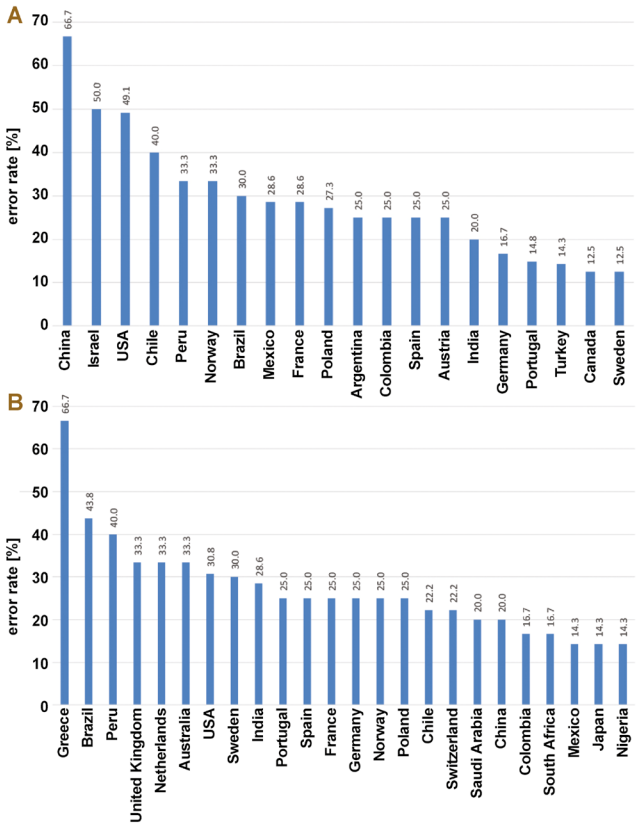


Fig. 8. Distribution of error rates by country for question 1

A. D-Ds; B. S-DHs.

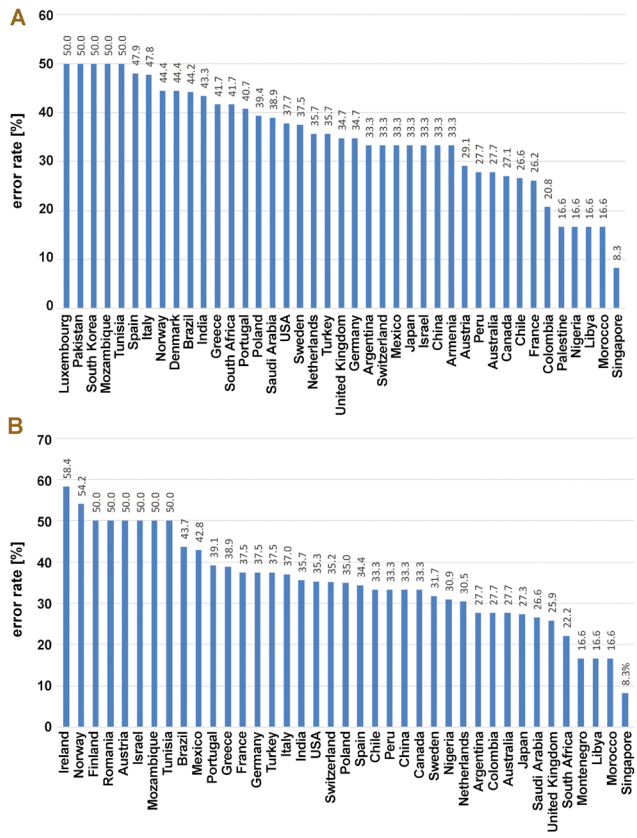


Fig. 9. Distribution of error rates by country for question 2

A. D-Ds; B. S-DHs.

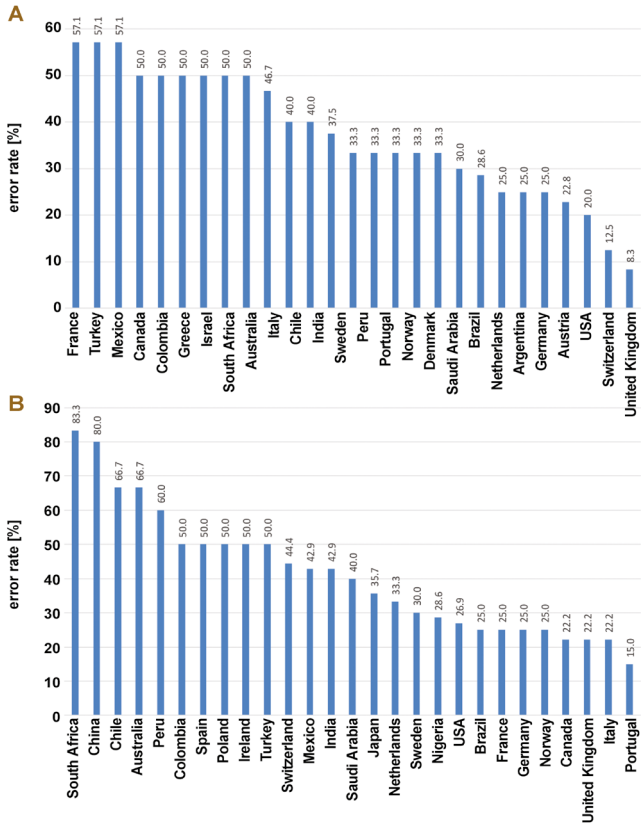


Fig. 10. Distribution of error rates by country for question 3

A. D-Ds; B. S-DHs.

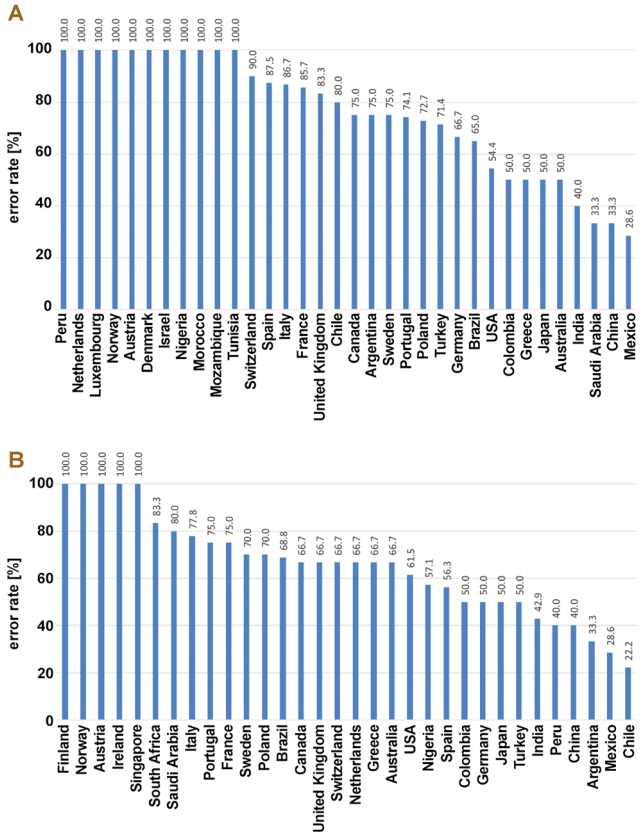


Fig. 11. Distribution of error rates by country for question 4

A. D-Ds; B. S-DHs.

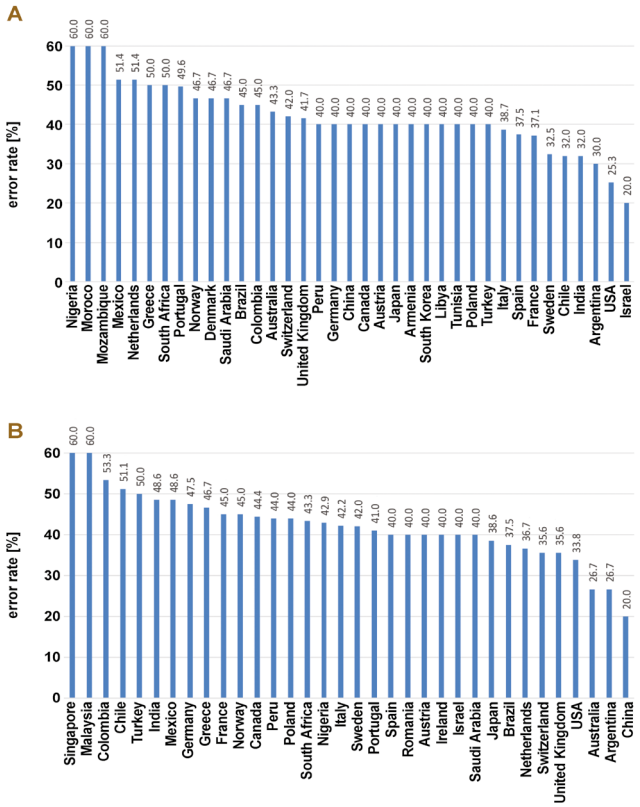


Fig. 12. Distribution of error rates by country for question 5

A. D-Ds; B. S-DHs.

Pearson correlation coefficients (r) between question scores are presented in Table 8. This coefficient ranges from –1 (perfect negative correlation) to 1 (perfect positive correlation), with 0 indicating no linear relationship. The correlation matrix revealed distinct clustering patterns and relationships among the questions, as follows:

- strong positive correlations:
  - Q1 & Q2 (r = 0.73): countries with high error rates for Q1 also tended to have high error rates on Q2, suggesting that these questions may reflect similar underlying knowledge domains or cognitive skills;
  - Q2 & Q5 (r = 0.68): performance on Q2 was a good predictor of the performance on Q5, and vice-versa;
  - Q4 & Q5 (r = 0.71): the strongest correlation among the latter questions. Countries with high error rates for Q4 also tended to demonstrate high error rates for Q5, indicating that the questions were closely linked in terms of required knowledge;
- moderate positive correlations:
  - Q1 & Q5 (r = 0.52): a moderate link exists between the first and final questions;
  - Q1 & Q4 (r = 0.44) and Q2 & Q4 (r = 0.42): these results suggest a tentative connection between the knowledge tested in the first 2 questions and that in Q4;
- weak or no correlations:
  - Q3 & all other questions (r < 0.4): Q3 demonstrated the weakest relationships with all other questions, with r-values of 0.31, 0.25, 0.18, and 0.37. This critical finding indicates that Q3 measured a distinct and independent knowledge domain. A country's performance on Q1, Q2, Q4, or Q5 is a very poor predictor of its performance on Q3.

Inter-question correlation

The inter-question correlation matrix demonstrated a more complex interaction among questionnaire items when analyzed at the participant level (Fig. 13). Unlike the continent-level analyses, several question pairs produced negative correlations, indicating that participants who answered one question incorrectly were more likely to answer another correctly.

Negative associations were especially prominent for Q3, which correlated inversely with Q2 (r = –0.63), Q4 (r = –0.66) and Q5 (r = –0.92). These strong negative relationships suggest that Q3 assesses a concept or skill fundamentally distinct from the others, such that misunderstanding in that domain may coincide with greater accuracy elsewhere. This pattern indicates that participants who struggled with Q3 often performed better on questions more closely linked to procedural or technical diagnostic components.

Table 8. Pearson correlation matrix of question-specific error rates among the 10 countries with the highest overall error rates

Question	Q1	Q2	Q3	Q4	Q5
Q1	1.00	–	–	–	–
Q2	0.73*	1.00	–	–	–
Q3	0.31	0.25	1.00	–	–
Q4	0.44	0.42	0.18	1.00	–
Q5	0.52	0.68*	0.37	0.71*	1.00

* strong positive correlation.

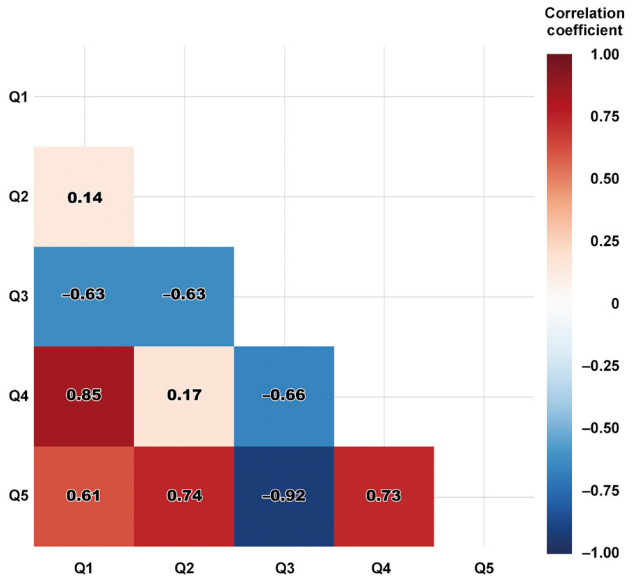


Fig. 13. Heatmap visualization of the inter-question correlation matrix

Conversely, Q4 showed a strong positive association with Q1 (r = 0.85), as well as moderate positive relationships with Q2 (r = 0.17) and Q5 (r = 0.73). This pattern indicates that Q4 clustered with items relying on similar reasoning patterns or conceptual frameworks. Questions 1, 4 and 5 formed a distinct, positively correlated cluster ($r \approx 0.61–0.85$), reflecting alignment among precision-probing practices, the perceived importance of PD and radiographic interpretation strategies. Collectively, these items represent technical aspects of diagnosis. Their positive associations imply that competence or conceptual clarity in one of these domains tends to coincide with similar accuracy in the others.

Taken together, these results reveal 2 major latent structures: cluster involving Q1, Q2, Q4, and Q5, which tend to rise and fall together; and another cluster centered on Q3, which behaves inversely relative to the remaining questions. This two-cluster structure suggests that the questionnaire captured at least 2 distinct underlying competencies or misconception profiles.

Overall, the observed patterns indicate the presence of at least 2 latent constructs underlying clinician diagnostic behavior: a technical/procedural dimension involving probing measurement, parameter prioritization and radiographic interpretation; and a cognitive-judgment dimension involving integration of clinical experience with formal guidelines.

Discussion

The present study explored basic concepts of periodontal assessment, aiming to evaluate global baseline knowledge in periodontics and to identify common diagnostic patterns and clinical standards. Overall, the survey indicates that, despite substantial variability in training backgrounds and practice settings, clinicians largely adopt guideline-concordant, evidence-based approaches to periodontal diagnosis. Respondents favored conservative, reproducible probing strategies – either rounding measurements up or down or recording them as read – rather than overestimating disease severity (Q1), and consistently prioritized the triad of RBL, PD and CAL as essential diagnostic parameters (Q2). Most participants reported following contemporary classification systems, particularly the 2017 AAP/EFP framework (Q3),[22] while recognizing PD as relevant but not a standalone or primary metric for detecting periodontitis (Q4), instead placing it within a broader diagnostic context. Interestingly, Q4 elicited the highest proportion of incorrect responses, with many participants rating PD as equally important as CAL and RBL. Radiographic assessment (Q5) was also approached in an integrated and systematic manner, combining imaging with probing data and emphasizing the evaluation of bone loss patterns and the relationship between the alveolar crest and CEJ, especially on bitewing radiographs. However, about half of the participants did not correctly interpret radiographic images for the diagnosis of periodontitis.

Although the survey response rate of 16.32% exceeded the target sample size required for the desired margin of error, it raises important considerations regarding selection bias and the generalizability of the results. The low response rate suggests that the 543 participants who completed the survey may represent a random sample of the 3,328 invited professionals. This introduces the potential for selection bias, which can impact the generalizability of the findings. In voluntary surveys, professionals who choose to participate are often those with greater interest in the subject matter (periodontics) or higher confidence in their knowledge. Consequently, respondents may have been more familiar with contemporary guidelines, such as the 2017 AAP/EFP classification system, and more competent in their diagnostic skills. In the presence of competence bias, the overall error rates observed (e.g., a mean overall score of 39.8%) may underestimate the actual global knowledge deficits and technical inconsistencies among the broader population of invited practitioners. Moreover, the impact on group comparisons, demonstrating that S-DHs consistently outperformed D-Ds, might be amplified if, for example, the D-Ds who responded were the least engaged in their group, while the S-DHs were the most engaged. Furthermore, influences on observed heterogeneity can introduce substantial variability in error rates across continents, countries and professional groups, and these differences are likely genuine. Still, their magnitude may differ in the overall population. The structured nature of the observed error patterns suggests that the underlying issues are real; however, the precise percentage differences may shift if a larger, more representative sample were obtained. In summary, while the sample size was statistically sufficient for the study's primary goal (descriptive prevalence estimates at ±5% margin of error), the low response rate indicates that the results are most accurately generalizable to the subset of globally invited, more engaged professionals who chose to complete the questionnaire, rather than to the entire population of invited experts. Future studies should, therefore, aim to achieve higher participation rates to validate these findings.

Taken together, the findings of the present study suggest that current periodontal diagnostic behavior among most respondents is broadly in line with accepted practices. At the same time, they highlight the need to reinforce, disseminate and implement standardized diagnostic protocols globally and to build upon the existing foundation to refine future educational and calibration efforts.[1,11,19]

A critical dual-level analysis provided further insight into how clinicians conceptualize and apply diagnostic principles. The strong positive correlations among continents demonstrate that, across widely disparate educational systems and clinical cultures, shared difficulties in basic diagnostic tasks exist. The clustering of questions 1, 2 and 5 suggests that uncertainty in probe-based

measurements, selection of essential diagnostic parameters, and radiographic evaluation are not isolated problems but interconnected elements of a global diagnostic pattern. These results indicate that technical inconsistencies in basic clinical procedures remain an entrenched problem. For example, the high intercontinental concordance in responses to Q1 indicates that uncertainty regarding probe markings, such as interpretation of 3.1–3.2-mm readings, is common worldwide. Similarly, the linked responses to questions 2 and 5 suggest persistent confusion regarding the diagnostic parameters clinicians consider most important and the interpretation of radiographic findings in relation to those parameters. This global synchrony indicates that efforts to standardize education might have a far-reaching impact.[1]

In contrast, the weaker association of Q4 with the other items introduces an important nuance: although PD is an important clinical variable, it should remain subordinate to CAL and RBL in the classification of periodontitis.[1] The data suggests that clinicians do not uniformly conceptualize PD in this hierarchical manner and may underestimate the influence of gingival inflammation, which could lead them to overestimate PD and, by extension, the severity of the disease. Supporting this interpretation, questions 3 and 4 are moderately related, suggesting that perceptions regarding the importance of PD are partly determined by broader diagnostic philosophy – that is, how clinicians trade off reliance on formal guidelines against personal clinical judgment. This relative independence may reflect the current debate surrounding the interpretation of PD in relation to CAL and RBL.[1,23]

At the individual level, the negative correlations involving Q3 further underscore this philosophical divide. Respondents who found the question about balancing clinical judgment with evidence-based guidelines challenging tended to perform better on the more technical questions. This could indicate a subgroup of clinicians who confidently apply procedural skills but whose diagnostic practice is less aligned with normative diagnostic frameworks, including the 2017 classification system.[22,23,26] In contrast, those who demonstrated conceptual clarity on Q3 may have been more critical or nuanced in interpreting the technical items, thereby creating different response patterns.

This divergence underscores a central tension in modern periodontal diagnosis: the integration of fine-grained clinical reasoning with standardized, guideline-driven pathways. Diagnostic competence cannot easily be placed along a single continuum; rather, the findings suggest a bifurcation into procedural competence and conceptual reasoning. Consequently, strategies for improving periodontal education should address both domains simultaneously.[11] Training that enhances measurement precision but does not place the measurements within an appropriate conceptual framework cannot be expected to ameliorate the variability described in this study.

Taken together, these results suggest that clinicians' diagnostic performance is the product of related but separable competencies. Correlation analyses support a two-construct model of diagnostic performance and underscore the need for focused training that aligns technical accuracy with evidence-based decision-making. Such findings have implications for more integrated education strategies that harmonize worldwide clinical practice with current periodontal standards while minimizing residual diagnostic disparities.

Geographical patterns further contextualize these observations. Questions 1, 3 and 4 demonstrated the greatest intercontinental dispersion. Africa and Oceania were consistently among the highest-error regions, while Europe and Asia were continuously in the mid-range. These trends suggest that diagnostic performance depends not only on individual training level but also on wider geographic and educational contexts, reinforcing the global heterogeneity identified at the country level. Clear differences were also observed between professional groups. The median error rates for S-DHs were consistently lower across countries, while those for D-Ds were more dispersed and exhibited more extreme outliers. Intercountry heatmaps revealed clusters of higher- and lower-performing countries. The results support the notion that national educational backgrounds shape diagnostic decision-making. Three principal patterns emerged from this data: S-DHs tended to outperform D-Ds across most diagnostic tasks; specific questions, particularly Q1 and Q5, highlighted striking educational or conceptual gaps; and diagnostic performance varied markedly across countries, reflecting the influence of varied training models, clinical guidelines and professional cultures.

Several factors limit this study. The number of participants per country was limited, potentially affecting the generalizability of comparisons at the country level. Moreover, the reliability and self-reported nature of the responses could not be fully verified. These limitations should be considered when interpreting findings and planning future, more comprehensive investigations.

This global analysis has demonstrated striking variation in periodontal diagnostic performance across continents, countries and professional groups; however, the observed pattern of errors was structured rather than random. Specialists and dental hygienists consistently outperformed general dentists and students, especially on questions requiring deeper interpretation. Correlation analyses indicated that most mistakes clustered within a shared technical domain – PD precision, selection of diagnostic parameters and radiographic interpretation – while the item addressing the balance between clinical judgment and evidence-based guidelines emerged as a separate, judgment-based construct. Collectively, these findings support a dual-competency model in periodontal diagnosis in which both technical/procedural skills and interpretive, guideline-based reasoning require reinforcement through education and calibration programs.

At the country level, the lowest-performing countries combined strong core knowledge, as reflected in Q1, Q2, Q4, and Q5, with a more independent domain assessed by Q3. The lowest 10 performances tended to accumulate errors across multiple technical items and showed weaker integration of contemporary classification systems and evidence-based frameworks into the participants' responses, suggesting systemic gaps in training, dissemination of clinical guidelines or calibration opportunities.

Conclusions

Within the limitations of this study, the findings suggest that improving global knowledge of periodontal diagnosis will require coordinated efforts that balance technical calibration with the consistent adoption of modern diagnostic guidelines, especially in lower-performing regions.

To address the observed global disparities and the dual-competency gap in periodontal diagnosis, future educational strategies must simultaneously harmonize technical skills with conceptual reasoning. Several core areas warrant particular attention:

- enhancing technical calibration (procedural competence) with simulation-based training for PD precision: implementing "gold standard" calibration workshops using periodontal manikins with predefined measurements. Clinicians should repeatedly perform assessments until their readings consistently fall within a narrow tolerance range relative to the reference values, focusing on conservative strategies such as rounding down or recording exact measurements;
- standardized radiographic protocol training: implementing training modules focused on bitewing radiographs as the preferred method for the assessment of the alveolar bone crest relative to the CEJ, and further utilization of computer-assisted diagnostic systems and AI algorithms to provide instant feedback on manual RBL interpretations, minimizing human error and standardizing the process;
- technology integration for accuracy: integrating digital periodontal probes with pressure sensors to provide real-time feedback on correct probing force, thereby reducing the risk of inflated PD readings. Advanced software or 3D imaging (cone beam computed tomography (CBCT)) may further assist clinicians in visualizing vertical and horizontal bone loss patterns and furcation involvement that are difficult to interpret on 2D images;
- hierarchical case-based reasoning: education must shift toward using real-world case studies to demonstrate the hierarchical importance of diagnostic parameters explicitly. Clinical attachment level should be considered the most essential metric, followed by RBL, and then PD. Training should emphasize diagnosis primarily based on CAL and RBL, while positioning PD mainly as a monitoring parameter rather than a standalone metric for periodontitis;
- conceptual and philosophical integration: seminars should be used to facilitate discussion and help clinicians reconcile their personal clinical experience and judgment with formal evidence-based guidelines, such as the 2017 AAP/EFP classification system. The goal should be to contextualize clinical judgment within the standardized framework, bridging the central tension between technical confidence and guideline adherence;
- mandatory global dissemination: standardized continuing education programs focused on contemporary classification systems should be promoted internationally, especially in lower-performing regions identified in the present study. Implementing train-the-trainer models can accelerate the dissemination of consistent, evidence-based knowledge through local professional organizations worldwide.

Collectively, these coordinated efforts may strengthen both technical/procedural competence and interpretive, guideline-based reasoning, ensuring a more reliable and ethically sound global standard for periodontal practice.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Periodontal symptoms, immunometabolic factors and autoimmune diseases: A prospective cohort study

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Conflict of interest
None declared

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Abstract

Background. Periodontal health is increasingly recognized as an integral component of systemic homeostasis. However, whether its disruption contributes to the development of autoimmune diseases (ADs) remains unclear in prospective settings.

Objectives. The aim of this study was to prospectively evaluate the association between periodontal symptoms and the risk of ADs, with particular attention to immunometabolic pathways and shared disease clusters.

Material and methods. A total of 465,454 participants from the UK Biobank were included. Cox proportional hazards models were employed to evaluate the association between periodontal symptoms and the incidence of 20 ADs. Sensitivity and subgroup analyses were conducted to assess the robustness of the results. Mediation analyses explored the potential mediating roles of 6 immunometabolic biomarkers. To address inter-disease correlations and reduce multicollinearity, factor analysis was applied to identify latent clusters of ADs.

Results. During a median follow-up of 13.3 years, 33,895 participants developed at least 1 AD. Periodontal symptoms were linked to a higher risk of several ADs, with significant associations observed for bullous disorders, lichen planus, primary biliary cholangitis (PBC), and psoriasis. Mediation analyses revealed modest but statistically significant contributions of immunometabolic markers, particularly C-reactive protein (CRP). Factor analysis identified 3 AD clusters – autoantibody-mediated, autoimmune inflammatory and autoimmune metabolic diseases – all of which were significantly associated with poor periodontal health.

Conclusions. Periodontal symptoms were positively associated with multiple ADs and latent clusters, with these correlations being partly mediated by immunometabolic factors. The findings highlight the role of periodontal–systemic interactions in autoimmune pathogenesis.

Keywords: C-reactive protein, autoimmune disease, periodontal health, prospective cohort, periodontal symptoms

Highlights

- This 13-year prospective cohort study involving approx. 500,000 individuals investigated the association between periodontal symptoms and autoimmune diseases.
- The analysis explored the potential mediating roles of 6 immunometabolic markers.
- Distinct clusters of autoimmune diseases were identified in association with periodontal symptoms.
- The findings emphasize periodontal health as a modifiable factor in autoimmune prevention.

Introduction

Autoimmune diseases (ADs) comprise a heterogeneous group of immune-mediated disorders characterized by the loss of self-tolerance and progressive tissue damage.[1] Affecting approx. 10% of the global population, ADs represent a growing public health concern due to their considerable morbidity, long-term functional impairment and escalating healthcare burden.[2] Although the etiology of ADs is multifactorial, emerging evidence highlights the critical role of environmental exposures and immunometabolic disturbances in autoimmune pathogenesis.[3] Low-grade systemic inflammation, aberrant immune activation and metabolic dysregulation collectively disrupt immune homeostasis and contribute to autoimmunity.[4] However, the upstream drivers and modifiable risk factors underlying these mechanisms remain incompletely understood.

Periodontitis, ranked as the 6th most prevalent disease worldwide, is a chronic inflammatory condition triggered by oral microbiota dysbiosis and sustained by dysfunctional host immune responses.[5] Numerous epidemiological studies have demonstrated associations between periodontitis and systemic conditions, including cardiovascular disease (CVD), cognitive impairment, cancer, and iodine deficiency.[6–10] Increasingly recognized as a systemic condition rather than a localized oral pathology, periodontitis exerts far-reaching effects on immune regulation and metabolic balance.[11] Through the oral–systemic axis, translocation of pathogenic microbes and persistent periodontal inflammation foster chronic immune activation, which may lead to insulin resistance, lipid abnormalities and impaired glucose regulation.[12] These convergent disruptions define a state of immunometabolic dysregulation – a central pathway increasingly implicated in the development of various systemic diseases, including ADs.[13]

A growing body of epidemiological research has reported correlations between periodontal disease and an increased risk of ADs. Meta-analyses have reported that periodontitis is associated with both increased prevalence and higher incidence of ADs.[14,15] This link is thought to be driven, at least in part, by shared immunometabolic pathways: chronic periodontal inflammation may disturb systemic immune–metabolic equilibrium and promote autoreactive responses.[16,17] Moreover, the relationship appears to be bidirectional, as autoimmune activity may also exacerbate periodontal breakdown via overlapping inflammatory cascades.[18,19] Supporting this, clinical trials have demonstrated that periodontal therapy can reduce systemic inflammatory markers and disease activity in patients with ADs.[20]

Periodontal symptoms, often the primary reason for dental consultations, reflect not only local disease but also systemic health status, as they are associated with inflammation, nutritional status and cardiometabolic disorders.[21,22] Notably, these symptoms represent modifiable and controllable factors, making them promising targets for preventive strategies against ADs. However, most existing evidence is derived from cross-sectional studies, leaving causal relationships and underlying mechanisms insufficiently defined. To address this gap, we conducted a large-scale prospective cohort study to (1) evaluate the association between periodontal symptoms and incident ADs, (2) assess the potential mediating role of immunometabolic pathways, and (3) identify clusters of ADs associated with periodontal symptoms. These findings may enhance understanding of oral–systemic interactions and support the early identification and prevention of ADs.

Material and methods

Study design

This study was based on the data obtained from the UK Biobank, a prospective cohort comprising over 500,000 participants aged 40–69 years who were recruited through the National Health Service (NHS).[23] The UK Biobank received ethics approval from the North West Multi-centre Research Ethics Committee (approval No. 11/NW/0382), and written informed consent was obtained from all participants. The present analysis was conducted in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.[24] Of the 502,415 participants enrolled in the UK Biobank, individuals who had withdrawn consent (n = 48), had missing data (n = 23,816) or had been diagnosed with an AD prior to baseline (n = 13,097) were excluded, resulting in a final analytical sample of 465,454 participants. Primarily, the association between periodontal symptoms and the incidence of 20 ADs was examined. Subgroup and sensitivity

analyses were performed to evaluate the robustness of the results. For ADs with significant associations, the potential mediating roles of 6 representative immunometabolic biomarkers were further investigated. Finally, factor analysis was applied to identify latent clusters within the AD spectrum (Fig. 1).

Assessment of periodontal symptoms

Baseline periodontal symptoms were defined using self-reported oral symptoms collected through a touchscreen questionnaire administered during the initial assessment visit. Participants were asked the following question: "Do you have any of the following? (select all that apply)", with response options including "bleeding gums", "painful gums" and "loose teeth". These patient-reported outcomes are widely used in epidemiological research and are considered clinically meaningful proxies for periodontal health.[25–27] Hospital-diagnosed periodontal disease, defined as a recorded diagnosis of gingivitis or periodontitis at study entry based on the International Classification of Diseases, Ninth or Tenth Revision (ICD-9/10) codes, was used as a supplementary measure. As the UK Biobank does not collect direct clinical measures of periodontal status (e.g., clinical attachment loss or pocket depth), the dual-source definition of both self-reported and clinically diagnosed data may help minimize misclassification and strengthen the validity of periodontal health assessment (Table S1 (available on request from the corresponding author)).

Assessment of autoimmune diseases

Autoimmune diseases were identified from the UK Biobank hospital records using the ICD-9/10 codes, with composite definitions applied to capture clinically relevant phenotypes (Table S2). Follow-up spanned from baseline until the first AD diagnosis, death, loss to follow-up, or the end of the follow-up period (April 2024), whichever occurred first. The present analysis included 20 conditions: ankylosing spondylitis; autoimmune hepatitis; autoimmune thyroiditis; bullous disorders; celiac disease; Crohn's disease; immune thrombocytopenic purpura (ITP); lichen planus; multiple sclerosis; myasthenia gravis; myositis; primary biliary cholangitis (PBC); psoriasis; rheumatoid arthritis; rheumatic heart disease (RHD); sarcoidosis; sicca syndrome; systemic lupus erythematosus (SLE); type 1 diabetes; and ulcerative colitis.

Measurement of mediators

Baseline blood samples were processed for biochemical analysis to quantify 6 immunometabolic markers involved in inflammation, lipid metabolism and glucose regulation: C-reactive protein (CRP), an acute-phase marker of systemic inflammation; triglycerides and high-density lipoprotein cholesterol (HDL-C), representing opposing lipid effects on cardiometabolic risk; low-density lipoprotein cholesterol (LDL-C), an atherogenic lipid associated with inflammation and metabolic dysfunction; glycated hemoglobin (HbA1c), a marker of long-term glycemic control; and the triglyceride–glucose (TyG) index, a surrogate marker of insulin resistance integrating lipid and glucose metabolism (Table S3). These markers were chosen as potential mediators linking periodontal disease to autoimmune outcomes due to their established roles in modulating immune responses and disease progression through systemic inflammation, lipid regulation and glycemic control.[28–30]

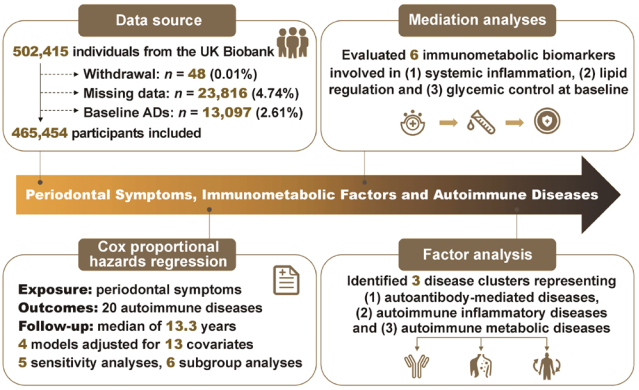


Fig. 1. Flowchart of study design

Measurement of covariates

Baseline covariates included age, sex, ethnicity, body mass index (BMI), Townsend deprivation index (TDI), smoking status, alcohol consumption, physical activity, healthy diet score, and a history of CVD, diabetes, hypertension, and depression (Table S3). Body mass index was calculated as body weight in kilograms divided by height in meters square (kg/m^2). The TDI was used as an area-level indicator of socioeconomic status, with higher values indicating greater socioeconomic deprivation. Smoking status, alcohol intake and physical activity were self-reported using baseline questionnaires. The healthy diet score was constructed according to dietary guidelines from the American Heart Association (AHA), with higher scores reflecting greater adherence to recommended dietary patterns.

Statistical analysis

The association between baseline periodontal symptoms and incident ADs was assessed using Cox proportional hazards regression models. Model 1 was the absolute risk model, while model 2 adjusted for demographic variables, including age, sex and ethnicity. Model 3 further adjusted for socioeconomic and lifestyle factors, including educational attainment, TDI, BMI, smoking status, alcohol intake, physical activity, and healthy diet score. Model 4

additionally adjusted for baseline comorbidities, including CVD, type 2 diabetes mellitus, hypertension, depression, cancer history, and medication use. Hazard ratios (*HR*s) and 95% confidence intervals (*CI*s) were calculated to estimate relative risk. Person-time was computed from baseline to the earliest occurrence of AD diagnosis, death, loss to follow-up, or the end of follow-up.

Sensitivity analyses excluded participants diagnosed with ADs within the first 2 years of follow-up, individuals of non-European ancestry, and those with comorbidities at baseline. To examine the influence of periodontal disease severity, bleeding gums were considered as a surrogate marker of mild disease, whereas loose teeth or painful gums served as surrogate markers of severe disease. Subgroup analyses were stratified by sex (male/female), age group (<50, 50–60, >60 years), physical activity (regular/irregular), smoking status (never, former, current), alcohol intake (never, former, current), and BMI category (underweight/normal: <25 kg/m^2, overweight: 25–30 kg/m^2, obese: >30 kg/m^2).

Mediation effects were estimated using counterfactual-based models with 1,000 bootstrap resamples. The average causal mediation effect (ACME) quantified the indirect effect mediated by the variable, whereas the average direct effect (ADE) represented the remaining direct effect. The proportion mediated (Prop) indicated the share of the total effect explained by the mediator.

To address multicollinearity among ADs, factor analysis was performed using a tetrachoric correlation matrix. Sampling adequacy was assessed with Bartlett's test of sphericity and the Kaiser–Meyer–Olkin (KMO) statistic. Latent factors were extracted via principal axis factoring, and the optimal number of factors was determined through parallel analysis and scree plot inspection. Clinically interpretable factor scores were converted into regression scores and analyzed as continuous outcomes in Cox proportional hazards regression models to capture underlying disease patterns.

All analyses were performed using R software (v. 4.4.3; R Foundation for Statistical Computing, Vienna, Austria). Two-sided *p*-values <0.05 adjusted for false discovery rate (FDR) were considered statistically significant.

Results

After excluding individuals with missing data (4.74%), a prior history of ADs (2.61%), and those who withdrew from the study (0.01%), 465,454 participants were included in this prospective cohort study. The mean age at baseline was 56.46 ±8.09 years; 54.3% of participants were female and 95.0% were of European ancestry. During a median follow-up of 13.3 years, 33,895 participants (7.3%) developed at least 1 AD of interest. Compared with those who remained free of ADs, affected individuals were older, had higher educational attainment, and were more likely to reside in socioeconomically deprived areas. In contrast, individuals without ADs exhibited a more favorable baseline health and lifestyle profiles, including lower rates of smoking, more frequent alcohol consumption, and greater engagement in regular physical activity. Notably, periodontal health at baseline was significantly better among participants who did not develop ADs (Table 1).

In absolute risk models, periodontal symptoms were associated with increased risks of 6 ADs, namely autoimmune thyroiditis, lichen planus, PBC, psoriasis, RHD, and sicca syndrome. After adjustment for demographic variables, significant associations were observed for 10 ADs: ankylosing spondylitis; bullous disorders; ITP; lichen planus; PBC; psoriasis; RHD; rheumatoid arthritis; sicca syndrome; and type 1 diabetes. Following further adjustment for socioeconomic and lifestyle factors, 6 associations remained statistically significant. Periodontal symptoms were associated with increased risks of bullous disorders (*HR* = 1.42, 95% *CI*: 1.08–1.86, p_{FDR} = 0.022), ITP (*HR* = 1.27, 95% *CI*: 1.05–1.55, p_{FDR} = 0.029), lichen planus (*HR* = 1.55, 95% *CI*: 1.33–1.81, p_{FDR} < 0.001), PBC (*HR* = 1.51, 95% *CI*: 1.16–1.97, p_{FDR} = 0.006), psoriasis (HR = 1.18, 95% *CI*: 1.10–1.27, p_{FDR} < 0.001), and RHD (*HR* = 1.07, 95% *CI*: 1.01–1.13, p_{FDR} = 0.026). After adjusting for baseline comorbidities, some associations were attenuated; however, 4 remained statistically significant: bullous disorders (*HR* = 1.40, 95% *CI*: 1.07–1.84, p_{FDR} = 0.035); lichen planus (*HR* = 1.55, 95% *CI*: 1.32–1.81, p_{FDR} < 0.001); PBC (*HR* = 1.50, 95% *CI*: 1.15–1.96, p_{FDR} = 0.008); and psoriasis (*HR* = 1.17, 95% *CI*: 1.09–1.26, p_{FDR} < 0.001), suggesting that these correlations were robust and largely independent of baseline health conditions (Table 2). The cumulative hazard remained consistently higher among participants with poor periodontal health than among those with healthy periodontal status. However, for bullous disorders, this difference became apparent only after 10 years of follow-up, indicating a delayed association (Fig. 2).

Sensitivity analyses excluding participants of non-European ancestry (Table S4) and those diagnosed with ADs within the first 2 years of follow-up (Table S5) yielded similar findings. However, when analyses were restricted to individuals without baseline comorbidities (Table S6), only the correlation between periodontal symptoms and lichen planus remained statistically significant (*HR* = 1.60, 95% *CI*: 1.29–1.99, p_{FDR} < 0.001). The association profiles of ADs differed according to periodontal disease severity. Bleeding gums were used as a proxy for mild disease, while loose teeth or painful gums were considered surrogate markers of severe disease. The former were primarily associated with bullous disorders, ITP, lichen planus, PBC, and psoriasis (Table S7), whereas the latter showed stronger associations with a broader spectrum of conditions, including ankylosing spondylitis, lichen planus, PBC, psoriasis, RHD, rheumatoid arthritis, sicca syndrome, SLE, and type 1 diabetes (Table S8).

Table 1. Baseline characteristics of UK Biobank participants according to autoimmune disease status

Variable		Total (N = 465,454)	Autoimmune diseases	
			no (n = 431,559)	yes (n = 33,895)
Age *Me* ±*SD*		56.46 ±8.09	56.25 ±8.10	59.10 ±7.55
Sex, *n* (%)	female	252,757 (54.3)	234,287 (54.3)	18,470 (54.5)
	male	212,697 (45.7)	197,272 (45.7)	15,425 (45.5)
Ethnicity, *n* (%)	European	442,235 (95.0)	410,008 (95.0)	32,227 (95.1)
	other	23,219 (5.0)	21,551 (5.0)	1,668 (4.9)
BMI [kg/m^2] *M* ±*SD*		27.37 ±4.75	27.30 ±4.69	28.27 ±5.29
TDI *M* ±*SD*		−1.37 ±3.05	−1.39 ±3.04	−1.09 ±3.18
Education, *n* (%)	college	78,777 (16.9)	70,622 (16.4)	8,155 (24.1)
	other	154,645 (33.2)	145,698 (33.8)	8,947 (26.4)
	unknown	232,032 (49.9)	215,239 (49.9)	16,793 (49.5)
Smoking status, *n* (%)	never	256,201 (55.0)	240,002 (55.6)	16,199 (47.8)
	previous	161,211 (34.6)	147,636 (34.2)	13,575 (40.1)
	current	48,042 (10.3)	43,921 (10.2)	4,121 (12.2)
Alcohol intake, *n* (%)	never	19,101 (4.1)	17,310 (4.0)	1,791 (5.3)
	previous	15,800 (3.4)	14,087 (3.3)	1,713 (5.1)
	current	430,553 (92.5)	400,162 (92.7)	30,391 (89.7)
Physical activity, *n* (%)	regular	332,240 (71.4)	309,692 (71.8)	22,548 (66.5)
	irregular	133,214 (28.6)	121,867 (28.2)	11,347 (33.5)
Healthy diet score *M* ±*SD*		2.80 ±1.29	2.81 ±1.29	2.79 ±1.30
Periodontal health at baseline, *n* (%)	poor	83,447 (17.9)	77,119 (17.9)	6,328 (18.7)
	good	382,007 (82.1)	354,440 (82.1)	27,567 (81.3)
Cancer history, *n* (%)		163,454 (35.1)	151,099 (35.0)	12,355 (36.5)
Drug use, *n* (%)		16,796 (3.6)	15,130 (3.5)	1,666 (4.9)
CVD at baseline, *n* (%)		19,211 (4.1)	16,343 (3.8)	2,868 (8.5)
Depression at baseline, *n* (%)		3,976 (0.9)	3,490 (0.8)	486 (1.4)
Hypertension at baseline, *n* (%)		33,496 (7.2)	28,709 (6.7)	4,787 (14.1)
T2DM at baseline, *n* (%)		6,987 (1.5)	5,330 (1.2)	1,657 (4.9)
CRP [mg/L] *M* ±*SD*		2.51 ±4.20	2.43 ±4.06	3.49 ±5.51
HDL-C [mmol/L] *M* ±*SD*		1.45 ±0.38	1.46 ±0.38	1.40 ±0.39
LDL-C [mmol/L] *M* ±*SD*		3.57 ±0.87	3.58 ±0.86	3.44 ±0.90
Triglycerides [mmol/L] *M* ±*SD*		1.74 ±1.02	1.74 ±1.02	1.81 ±1.06
HbA1c [mmol/L] *M* ±*SD*		35.93 ±6.36	35.76 ±6.00	38.15 ±9.52
TyG index *M* ±*SD*		9.04 ±6.68	8.95 ±6.49	10.07 ±8.65

BMI – body mass index; CRP – C-reactive protein; CVD – cardiovascular disease; HbA1c – hemoglobin A1c; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; *M* – mean; *Me* – median; *SD* – standard deviation; TDI – Townsend deprivation index; T2DM – type 2 diabetes mellitus; TyG – triglyceride–glucose index. The healthy diet score was constructed according to dietary guidelines from the American Heart Association (AHA), with higher scores reflecting greater adherence to recommended dietary patterns.

Table 2. Association between periodontal health and the incidence of autoimmune diseases

Autoimmune disease	Person-years	Events, *n* (%)	Model 1[a]			Model 2[b]			Model 3[c]			Model 4[d]		
			HR	95% *CI*	p_{FDR}	*HR*	95% *CI*	p_{FDR}	*HR*	95% *CI*	p_{FDR}	*HR*	95% *CI*	p_{FDR}
Ankylosing spondylitis	6,194,751	774 (0.17)	1.16	0.97–1.38	0.178	1.28	1.07–1.53	0.009*	1.22	1.02–1.45	0.053	1.20	1.00–1.43	0.101
Autoimmune hepatitis	6,197,913	272 (0.06)	1.35	1.02–1.79	0.109	1.34	1.01–1.79	0.056	1.30	0.98–1.74	0.110	1.30	0.97–1.72	0.145
Autoimmune thyroiditis	6,193,683	997 (0.21)	1.24	1.06–1.44	0.025*	1.13	0.97–1.32	0.130	1.10	0.95–1.29	0.284	1.10	0.94–1.28	0.356
Bullous disorders	6,197,858	322 (0.07)	1.22	0.94–1.60	0.211	1.45	1.11–1.90	0.009*	1.42	1.08–1.86	0.022*	1.40	1.07–1.84	0.035*
Celiac disease	6,185,411	2,203 (0.47)	0.98	0.88–1.09	0.745	1.00	0.90–1.12	0.980	1.02	0.91–1.14	0.822	1.01	0.90–1.13	0.911
Crohn's disease	6,190,587	1,427 (0.31)	1.09	0.96–1.25	0.267	1.10	0.97–1.26	0.164	1.05	0.92–1.20	0.558	1.04	0.91–1.19	0.663
ITP	6,196,151	625 (0.13)	1.19	0.98–1.45	0.155	1.32	1.08–1.60	0.009*	1.27	1.05–1.55	0.029*	1.26	1.03–1.53	0.051
Lichen planus	6,194,512	873 (0.19)	1.52	1.31–1.78	<0.001*	1.56	1.34–1.82	<0.001*	1.55	1.33–1.81	<0.001*	1.55	1.32–1.81	<0.001*
Multiple sclerosis	6,192,782	998 (0.21)	1.03	0.87–1.21	0.745	0.97	0.83–1.14	0.748	0.96	0.81–1.12	0.673	0.94	0.80–1.11	0.598
Myasthenia gravis	6,197,926	289 (0.06)	0.87	0.63–1.19	0.442	1.02	0.74–1.40	0.908	0.96	0.70–1.32	0.853	0.96	0.70–1.31	0.848
Myositis	6,197,648	357 (0.08)	1.18	0.91–1.52	0.279	1.30	1.00–1.68	0.066	1.26	0.97–1.63	0.135	1.24	0.96–1.61	0.192
PBC	6,197,751	292 (0.06)	1.58	1.22–2.06	0.004*	1.59	1.22–2.07	<0.001*	1.51	1.16–1.97	0.006*	1.50	1.15–1.96	0.008*
Psoriasis	6,176,773	4,452 (0.96)	1.19	1.11–1.28	<0.001*	1.27	1.18–1.37	<0.001*	1.18	1.10–1.27	<0.001*	1.17	1.09–1.26	<0.001*
Rheumatoid arthritis	6,160,407	6,936 (1.49)	1.05	0.99–1.12	0.178	1.10	1.03–1.17	0.004*	1.04	0.98–1.10	0.331	1.02	0.96–1.09	0.611
RHD	6,166,588	9,324 (2.00)	0.92	0.88–0.98	0.024*	1.12	1.07–1.19	<0.001*	1.07	1.01–1.13	0.026*	1.05	1.00–1.11	0.124
Sarcoidosis	6,194,478	844 (0.18)	1.11	0.94–1.32	0.279	1.09	0.92–1.29	0.350	1.06	0.89–1.26	0.585	1.06	0.89–1.26	0.654
Sicca syndrome	6,194,415	878 (0.19)	1.25	1.06–1.46	0.025*	1.19	1.01–1.40	0.046*	1.18	1.00–1.39	0.078	1.16	0.99–1.37	0.135
SLE	6,196,373	529 (0.11)	1.21	0.99–1.50	0.153	1.16	0.94–1.43	0.188	1.12	0.90–1.38	0.399	1.10	0.89–1.35	0.554
Type 1 diabetes	6,184,303	2,525 (0.54)	1.10	1.00–1.21	0.153	1.18	1.07–1.30	0.002*	1.07	0.97–1.19	0.232	1.03	0.93–1.14	0.708
Ulcerative colitis	6,181,362	2,814 (0.60)	1.03	0.94–1.14	0.570	1.07	0.97–1.18	0.174	1.04	0.95–1.15	0.499	1.04	0.94–1.14	0.615

CI – confidence interval; FDR – false discovery rate; *HR* – hazard ratio; ITP – immune thrombocytopenic purpura; PBC – primary biliary cholangitis; RHD – rheumatic heart disease; SLE – systemic lupus erythematosus; [a] absolute risk model; [b] adjusted for age, sex and ethnicity; [c] further adjusted for education, TDI, BMI, smoking status, alcohol intake, physical activity, and diet score; [d] additionally adjusted for baseline CVD, T2D, hypertension, depression, cancer history, and drug use; * statistically significant (Benjamini–Hochberg-adjusted two-sided $p < 0.05$, Cox proportional hazards regression).

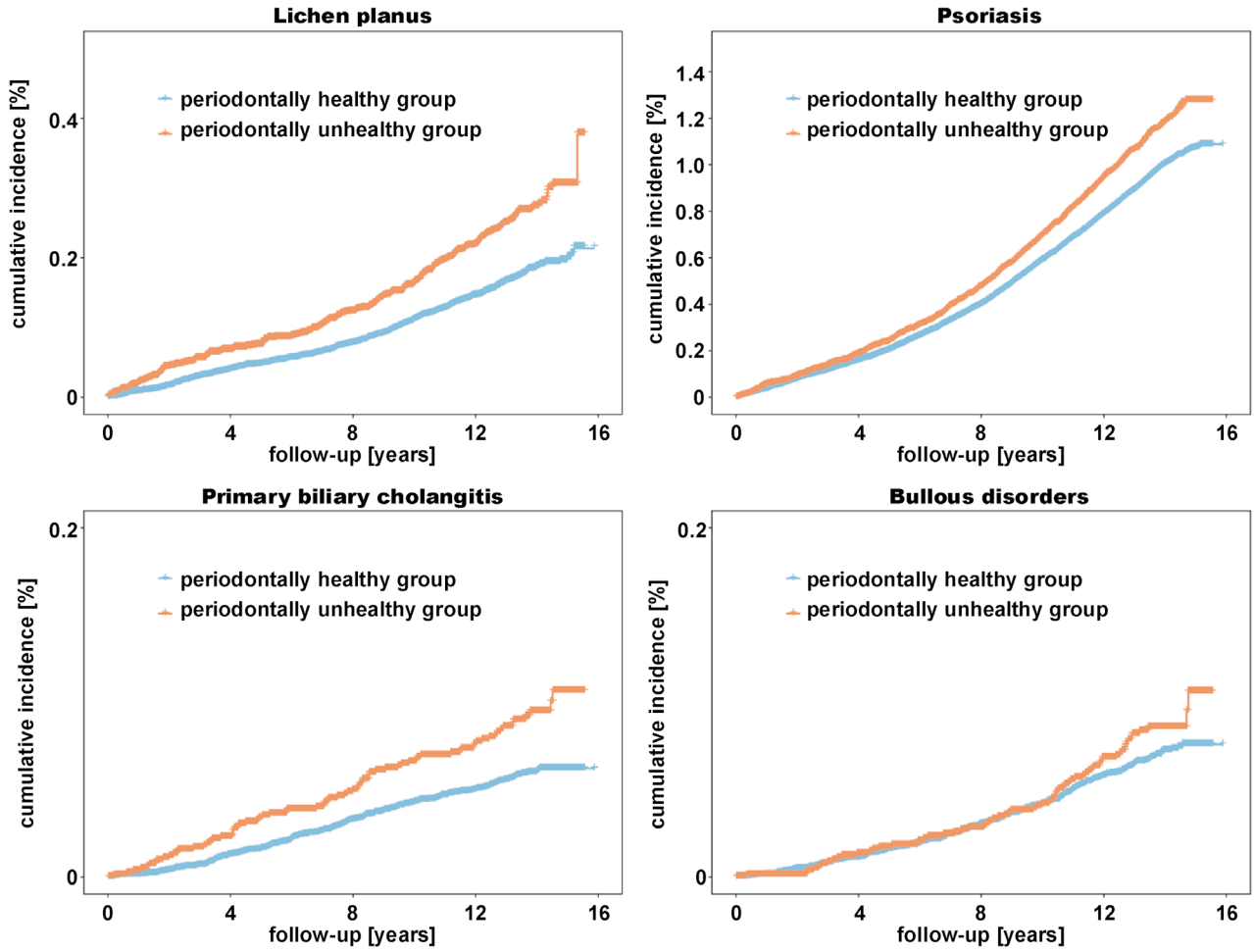


Fig. 2. Cumulative incidence of autoimmune diseases (ADs) during follow-up according to periodontal status

Subgroup analyses indicated significant age-related heterogeneity, particularly for the associations between periodontal symptoms and PBC (p for heterogeneity = 0.004) and rheumatoid arthritis (p = 0.003) (Table S9), with attenuated effect estimates observed in older age groups. Stratified analyses also suggested potential modification by alcohol intake and smoking status, with associations largely absent among never or former drinkers and among current or former smokers. In contrast, no significant effect modification was observed according to sex, BMI or physical activity, which may be attributable to limited statistical power within these subgroups (Table S10–S15).

To explore potential mechanisms, we assessed the mediating roles of 6 immunometabolic markers – CRP, HbA1c, HDL-C, LDL-C, triglycerides, and TyG – in the 4 robust periodontal–autoimmune associations. Overall, these biomarkers explained up to 1.9% of the observed associations, indicating modest but statistically significant mediation (Table S16). C-reactive protein consistently exhibited broad mediating effects, contributing to the associations between periodontal symptoms and bullous disorders (ACME: 1.88×10^{-6}, p = 0.006; Prop: 0.005, p = 0.012), PBC (ACME: 3.49×10^{-6}, $p < 0.001$; Prop: 0.009, p = 0.002) and psoriasis (ACME: 3.23×10^{-5}, $p < 0.001$; Prop: 0.019, $p < 0.001$). In addition, HbA1c significantly mediated the association between periodontal symptoms and lichen planus (ACME: 4.09×10^{-6}, p = 0.016; Prop: 0.003, p = 0.016). Triglycerides mediated a small proportion of the association between periodontal symptoms and PBC (ACME: -2.17×10^{-6}, p = 0.024; Prop: −0.006, p = 0.028), while TyG contributed to the association between periodontal symptoms and psoriasis (ACME: 9.00×10^{-6}, p = 0.014; Prop: 0.006, p = 0.014) (Fig. 3).

To address potential interactions and multicollinearity among ADs, factor analysis was performed for dimensionality reduction. Tetrachoric correlation matrices were computed to accommodate the binary nature of the outcomes. Bartlett's test of sphericity (χ^2 = 397.9, $p < 0.001$) and a KMO statistic of 0.57, although marginal, met the requirements for factor analysis. Parallel analysis and scree plot inspection supported a three-factor solution (Fig. 4A), yielding clusters representing (1) autoantibody-mediated diseases (e.g., SLE, sicca syndrome, rheumatoid arthritis), (2) autoimmune inflammatory diseases (e.g., Crohn's disease, ulcerative colitis) and (3) autoimmune metabolic diseases (e.g., PBC, autoimmune hepatitis) (Fig. 4B). Poor periodontal health was significantly associated with all 3 clusters (Fig. 4C). Sensitivity analyses using a two-factor

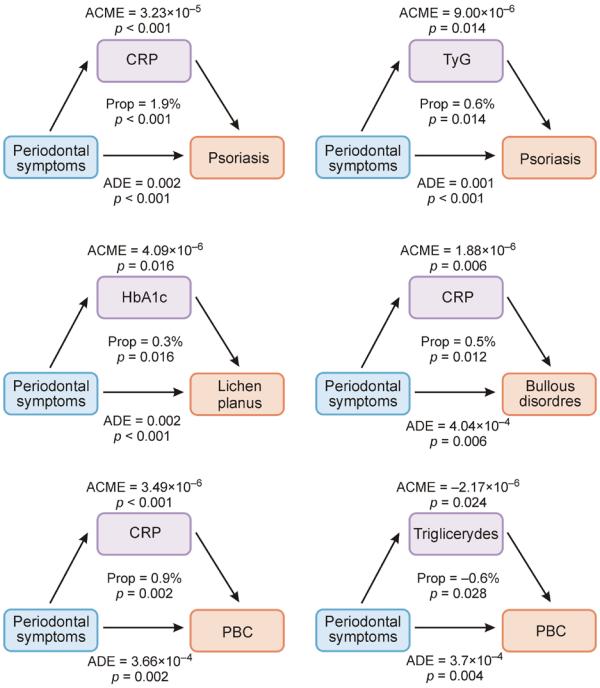


Fig. 3. Mediation analysis of immunometabolic markers on the associations between periodontal symptoms and the risk of autoimmune diseases (ADs)

ACME – average causal mediation effect; ADE – average direct effect; CRP – C-reactive protein; HbA1c – hemoglobin A1c; PBC – primary biliary cholangitis; Prop – proportion mediated; TyG – triglyceride–glucose index.

model – primarily combining autoantibody-mediated and autoimmune inflammatory diseases – yielded comparable results (Fig. S1, Table S17). To enhance model stability, 4 diseases with KMO values <0.5 were excluded, increasing the overall KMO to 0.71. This refined analysis identified an additional cluster of autoimmune muscle disorders. Associations between periodontal symptoms and all identified clusters remained significant (Fig. S2,S3, Table S18).

Discussion

Building on our previous research employing multi-omic genetic analyses to identify gene-level associations between ADs and specific oral manifestations, this study further investigated the immune-mediated oral–systemic axis.[31] In this large-scale prospective cohort study, we systematically evaluated the associations between periodontal symptoms and the incidence of 20 ADs among nearly half a million participants over a median follow-up of 13.3 years.

Our findings suggest a prospective association between poor periodontal health and an increased risk of several ADs. Although contemporary epidemiological research increasingly moves beyond a sole focus on statistical significance, 4 ADs – bullous disorders, lichen planus, PBC, and psoriasis – remained consistently and robustly associated with poor periodontal health across all adjusted models, warranting particular attention.[32] Mediation analyses revealed modest but statistically significant contributions from immunometabolic markers, particularly CRP, indicating that systemic inflammation may partially mediate the relationship between periodontal symptoms and autoimmune pathogenesis. In addition, factor analysis was applied to reduce dimensionality and cluster diseases, revealing a detrimental association between poor periodontal health and ADs.

The association between periodontal health and psoriasis has received considerable attention in both preclinical and clinical research. A meta-analysis of 13 studies reported a pooled odds ratio (*OR*) of 2.87 (95% *CI*: 1.75–4.69), indicating a strong association between periodontitis and psoriasis; however, the observational nature of these studies limits causal interpretation.[15] A recent randomized clinical trial further demonstrated that non-surgical periodontal therapy provided additional benefits beyond conventional dermatologic treatment in alleviating the severity of psoriasis.[33] Experimental studies in murine models support these findings, showing that induced periodontitis can trigger and sustain psoriasiform inflammation through systemic immune pathways.[34]

In addition, we identified significant associations between periodontal symptoms and several less-studied ADs, including bullous disorders, lichen planus and PBC. A systematic review has linked poor periodontal health with oral pemphigus vulgaris and mucous membrane pemphigoid, with improvements observed following oral hygiene interventions.[35] Similarly, a meta-analysis demonstrated that patients with oral lichen planus exhibited worse periodontal parameters.[36] However, these studies have been constrained by small sample sizes, heterogeneity and cross-sectional designs. By validating these associations in a large prospective cohort, our study provides stronger evidence supporting the potential relevance of periodontal health in systemic autoimmunity.

Inflammation is widely recognized as a key biological pathway potentially linking periodontitis to systemic disorders. Epidemiological studies have consistently reported associations between periodontitis and conditions such as prostatitis and gastroesophageal reflux disease, with chronic inflammation thought to mediate these relationships.[37,38] Initiated by persistent microbial exposure and dysregulated host responses, periodontitis promotes the release of pro-inflammatory cytokines that contribute to both local tissue destruction and systemic inflammation.[39,40] C-reactive protein, a sensitive biomarker of systemic inflammation, has been consistently associated with both autoimmune and cardiometabolic diseases.[41] Previous studies have shown that CRP levels are elevated in individuals with periodontitis and decrease following periodontal treatment.[42,43] A cross-sectional analysis further reported that the association between periodontitis and systemic diseases was amplified among individuals with elevated CRP levels.[44] Our findings support a partial

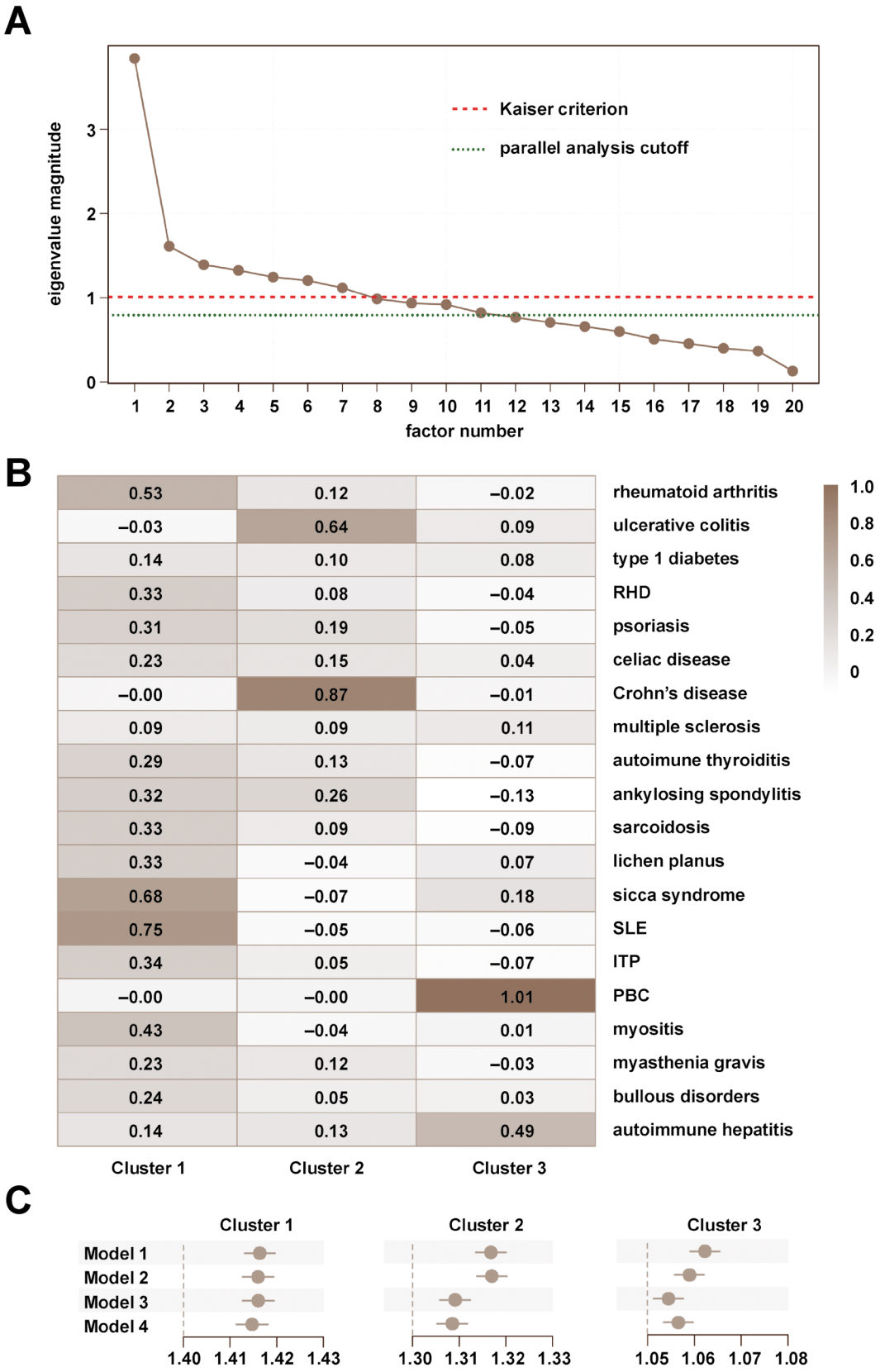


Fig. 4. Results of factor analysis

A. Scree plot indicating stabilization after the extraction of 3 factors; B. Factor loading matrix delineating 3 disease clusters: autoantibody-mediated diseases; autoimmune inflammatory diseases; and autoimmune metabolic diseases; C. Forest plots demonstrating strong positive associations between poor periodontal health and each AD cluster.
ITP – immune thrombocytopenic purpura; RHD – rheumatic heart disease; SLE – systemic lupus erythematosus.

mediating role of CRP in the associations between poor periodontal health and selected ADs, offering new insight into inflammation-driven oral–systemic interactions.

Emerging evidence suggests that metabolic dysregulation may represent a pivotal intermediary in the cross talk between periodontal symptoms and ADs. Periodontitis has been associated with metabolic disturbances, including insulin resistance and dyslipidemia.[45,46] In a large population-based study, tooth loss correlated with elevated very-low-density lipoprotein (VLDL)-related lipid fractions and triglyceride levels.[47] Similarly, a cross-sectional analysis reported a consistent association between the TyG index and periodontitis,[48] while Boyapati et al. identified CRP, LDL-C and HDL-C as key metabolic biomarkers of periodontal disease.[49] These metabolic abnormalities are not merely correlative but can actively reprogram immune responses by altering antigen presentation, impairing immune tolerance and fostering autoreactive phenotypes.[50,51] Moreover, among individuals with periodontitis, obesity has been associated with elevated serum leptin and decreased adiponectin levels.[52] Collectively, these findings support an emerging immunometabolic paradigm in which markers such as HbA1c, triglycerides and the TyG index may partially mediate the relationship between adverse periodontal profiles and increased AD risk.

Although epidemiological associations between periodontal health and ADs have been increasingly recognized, our study offers several novel contributions. First, by leveraging the UK Biobank, a large population-based prospective cohort comprising nearly 500,000 participants, we evaluated prospective associations between periodontal symptoms and ADs, providing evidence that may strengthen causal inference. Second, by acknowledging interrelated mechanisms underlying multiple ADs, we applied factor analysis to identify disease clusters and evaluate their aggregate associations beyond the level of individual diseases. Finally, given the limited understanding of the underlying mechanisms, we performed preliminary analyses of immunometabolic pathways, highlighting the potential role of immunometabolic factors in autoimmune pathogenesis.

Limitations

Several limitations should be considered when interpreting the findings. First, since the UK Biobank does not include direct clinical assessments of periodontal status (e.g., probing depth, attachment loss), we relied on self-reported symptoms, which are widely used and informative in large-scale studies.[25–27] Nevertheless, clinical dental examinations would provide greater diagnostic precision. Future studies should incorporate standardized periodontal assessments or interventional designs to strengthen causal inference and clinical applicability. Second, AD diagnoses relied on hospital ICD codes, a standard approach in epidemiological research; however, this method may fail to capture milder or undiagnosed cases that do not result in hospital-based care. Third, given the slow progression, shared genetic liability, complex pathophysiology, and frequent diagnostic delays characteristic of many ADs, reverse causality cannot be entirely excluded. Finally, although 6 representative immunometabolic biomarkers were included, they may not fully reflect the complexity of immune–metabolic interactions. The observed mediation effects were modest and should therefore be interpreted with caution, particularly in light of potential confounding from shared environmental or genetic factors.

Conclusions

Periodontal symptoms were positively associated with multiple ADs and their latent clusters, with particularly strong associations observed for bullous disorders, lichen planus, PBC, and psoriasis. These correlations were partly mediated by immunometabolic markers such as CRP, TyG, HbA1c, and triglycerides. Our findings suggest that periodontal health may represent a modifiable risk factor for ADs and underscore the importance of integrating oral health into broader public health strategies aimed at early prevention.

Ethics approval and consent to participate

The UK Biobank received ethics approval from the North West Multi-centre Research Ethics Committee (approval No. 11/NW/0382), and written informed consent was obtained from all participants.

Data availability

The original data can be obtained from the UK Biobank upon application (https://www.ukbiobank.ac.uk). The datasets supporting the findings of the current study, including supplementary materials, 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

Characteristics of orofacial function and sleep disorders in children with signs of autism spectrum disorder: A cross-sectional study

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Abstract

Background. Children with autism spectrum disorder (ASD) have higher rates of feeding problems and sleep disorders. Research on feeding problems in children with ASD has focused on behavioral, psychological and nutritional aspects. However, the biomechanics underlying these issues remains unknown.

Objectives. The present study aimed to evaluate the relationship between signs of ASD, orofacial function and sleep disorders in children.

Material and methods. A total of 56 Japanese early elementary school children aged 6–9 years were recruited. The subjects underwent physical examinations, intraoral inspections and orofacial functional assessments. The parents or legal guardians of the children completed questionnaires regarding the subjects' dietary and meal habits, the Social Responsiveness Scale, Second Edition (SRS-2), and the Japanese Sleep Questionnaire for Elementary Schoolers (JSQ-ES). Each assessment item was compared between 2 groups divided according to the SRS-2 scores, and the relationships with psychological traits were investigated using Spearman's rank correlation coefficient.

Results. Thirteen of the 56 children had SRS-2 T scores ≥60, indicating possible ASD. Masticatory performance was significantly lower in the high SRS-2 group than in the low SRS-2 group ($p < 0.05$). Additionally, the high SRS-2 group showed significantly higher T scores on the JSQ-ES for insomnia ($p < 0.05$) and excessive daytime sleepiness ($p < 0.01$). Occlusal force ($p < 0.05$), masticatory performance ($p < 0.05$), insomnia ($p < 0.001$), and excessive daytime sleepiness ($p < 0.001$) were significantly correlated with the SRS-2 score.

Conclusions. These results suggest that low masticatory performance contributes to feeding problems in children with signs of ASD. Additionally, the severity of ASD symptoms is associated with the severity of orofacial functional and feeding problems, as well as sleep disorders.

Keywords: autism spectrum disorder, sleep disorder, school children, orofacial function, masticatory performance

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Highlights

- Low masticatory performance, together with behavioral and psychological issues, may contribute to feeding difficulties in early elementary school children with signs of autism spectrum disorder (ASD).
- The severity of ASD symptoms is associated with impairments in orofacial function, feeding behavior and sleep.
- Targeted interventions addressing orofacial dysfunction and sleep disturbances are needed for children with signs of ASD.

Introduction

Autism spectrum disorder (ASD) is a common neurodevelopmental condition characterized by social and/or communication difficulties, as well as the presence of restrictive or repetitive behaviors.[1] Autism spectrum disorder has a worldwide prevalence of approx. 1–3% and occurs nearly 4 times more frequently in boys than in girls.[2] Previously, children with autism were diagnosed at ages older than 3–4 years. Currently, toddlers are more frequently diagnosed due to early recognition of atypical development.[3] However, high-functioning individuals are often diagnosed late or remain undiagnosed and latent.[3]

Autism spectrum disorder is frequently accompanied by sleep problems such as insomnia, circadian rhythm disorder and obstructive sleep apnea (OSA).[4] Approximately 60–80% of pediatric patients with ASD experience sleep difficulties, with insomnia being the most common.[5,6] As determined by actigraphy or polysomnography, children with ASD have reduced total sleep time, longer sleep latency and decreased sleep efficiency compared to typically developing children.[7] Additionally, children with ASD demonstrate higher rates of OSA, with a frequency of approx. 30–60%.[4,8] The severity of sleep problems in ASD is associated with the severity of ASD symptoms.[9]

Children with ASD are 5 times more likely to experience feeding problems than their peers, including unusual eating habits, feeding difficulties and restrictive diets.[10,11] Limited food preference is the most common feeding problem behavior in children with ASD, followed by hypersensitivity to food textures and eating only one brand of food.[11] Thus, research on feeding problems in children with ASD has focused on behavioral, psychological and nutritional aspects.[10–12] Additionally, pediatric patients with ASD frequently have hypotonia and apraxia, especially oral motor apraxia, manifesting as excessive drooling, an open mouth at rest, and an inability to pucker the lips, lick the lips with the tongue, or blow bubbles.[13] However, the majority of studies on oral motor skills in children with ASD have focused on speech.[14] Of the few publications addressing feeding, one reported significantly lower mylohyoid muscle activity among autistic children during eating.[14,15] Other studies have usually relied upon caregiver questionnaires. Therefore, further investigation of feeding problems associated with ASD in children, with a focus on the biomechanics of feeding, is warranted.

Mastication is a rhythmic movement regulated by a central pattern generator, and is the first step in the digestive process, in which food is broken down for swallowing.[16,17] The coordinated actions of the jaws, teeth, connecting muscles, lips, and tongue are critical in the mastication process.[18] Oral motor dysfunction, such as tongue protrusion, incoordination of eating and breathing, lack of rhythmic movement, and insufficient lip closure, can be associated with masticatory disabilities.[19,20] To evaluate masticatory function in children, masticatory performance is usually measured by determining the size of food particles after a standardized number of chewing cycles.[21] However, few studies have evaluated masticatory performance in children based on neurodevelopmental aspects.

Recent research has reported that improvements in orofacial function may contribute to enhanced sleep quality in postoperative residual OSA.[22] This indicates that orofacial dysfunction and sleep disorders could be mutually correlated in children with ASD. One study has recommended the incorporation of routine sleep screening into the clinical practice of general pediatric clinics, as there could be a specific subgroup of patients with sleep-disordered breathing (SDB) that may go undetected.[23] Therefore, using questionnaire-based assessments of SDB and ASD in pediatric dental practice may be useful to clarify the relationship between these 2 separate problems in children with ASD.

The present study aimed to assess the relationship between sleep problems, signs of ASD and orofacial function in children using screening tools. Additionally, the significance of interventions for these problems was discussed.

Material and methods

Ethics statement

The present study was conducted in strict accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Graduate School of Dentistry at the University of Osaka, Japan (approval No. R3-E5). All parents or legal guardians of the patients were informed of the content of the study and provided with written information as well as verbal explanations. Written informed consent for study participation was obtained from all parents/guardians, and verbal agreement and written informed assent were obtained from all children.

Participants

Fifty-six Japanese children aged between 6 and 9 years (38 boys and 18 girls; mean age: 7.8 ±0.9 years) who attended the Pediatric Dentistry Clinic at The University of Osaka Dental Hospital from October 2021 to August 2024 were recruited through announcements posted in the clinic. The participants had undergone regular oral health checks at the clinic; some had previously visited with primary complaints of dental caries, dental trauma, occlusion problems, and mesiodentes, while others had visited seeking regular oral care. The inclusion criteria encompassed children with normal language comprehension, no reported health problems (systemic diseases or soft tissue abnormalities including cleft lip and palate), completed caries treatment, no clinical diagnosis regarding neurodevelopmental or comorbid conditions, and no history of psychotropic medication use. Pediatric patients who were uncooperative during clinical examinations and those with incomplete questionnaires from parents/guardians were excluded. No participants declined to participate or dropped out of the study.

Psychological examination (symptom measurement)

The severity of ASD symptoms was assessed using the Social Responsiveness Scale, Second Edition (SRS-2), as reported by the parents or legal guardians of the participants.[24] Its predecessor, the SRS, has been one of the most frequently used quantitative measures of autism symptoms, with strong measurement properties in healthy and autism-affected samples.[25] For individuals aged 4–18 years, the SRS-2 represents the same item set as the SRS. The SRS-2 is an ordinally scaled (from 1 = not true to 4 = almost always true) quantitative assessment measuring 65 items regarding a child's behavior. The items are clustered into 5 subdomains: social awareness; social cognition; social communication; social motivation; and restricted repetitive behaviors. The discriminant validity of the SRS-2 is supported by sensitivity/specificity metrics and area under the curve (AUC) values across populations. The internal consistency (Cronbach's α) was 0.95 in the clinical sample and 0.97 in the non-clinical standardization sample, indicating high reliability. The total raw scores were assessed and converted into T scores, with values of 60–65, 66–75 and ≥76 indicating mild, moderate and severe ASD symptoms, respectively.

Examination of sleep disorders

The scores for sleep disorders were calculated using the Japanese Sleep Questionnaire for Elementary Schoolers (JSQ-ES), as reported by the parents or legal guardians of the participants.[26] The JSQ-ES is a validated screening tool designed to assess sleep problems in Japanese elementary school children. The JSQ-ES is an ordinally scaled (from 1 = strongly disagree/false/inapplicable to 6 = strongly agree/true/applicable) quantitative assessment measuring 38 items regarding sleep disorders and sleep habits in children. Exploratory and confirmatory factor analyses supported a nine-factor structure: restless legs syndrome; SDB; morning symptoms; nighttime awakenings; insomnia; excessive daytime sleepiness; daytime behavior; sleep habits; and irregular/delayed sleep phase. Scores clearly differentiated children with clinically diagnosed sleep disorders from the community sample, with an optimal cut-off score of 80 points. The internal consistency (Cronbach's α) was 0.88 in the community sample and 0.91 in the clinical sample, indicating high reliability. T scores and their sum in the 9 subdomains were assessed. Additionally, sleep bruxism was evaluated using an ordinal scale ranging from 1 to 6.

Questionnaires regarding dietary and meal habits

The following information was collected from participants' parents or legal guardians to evaluate dietary and meal habits: amount of food consumed per meal (excessive food intake, insufficient food intake, variable food intake); eating speed (too fast, too slow); picky eating (severe, mild, none); and behavior during meals (calm, standing up and playing).

The above 3 questionnaires were collected after the completion of the clinical examination and orofacial functional evaluation.

Clinical examination

All examinations were performed by a dentist (MK) under the supervision of a pediatric dentist with over 10 years of specialized experience (MO). The physical examinations included anthropometric measurements of height and weight. During the oral examinations, the number of erupted teeth and the total number of decayed, missing, and filled teeth (dmft/DMFT) were recorded by visual inspection with a dental mirror under lighting according to the criteria established by the World Health Organization (WHO).[27] Additionally, the number of teeth (deciduous + permanent teeth) and Hellman's dental age (IIA, IIC, IIIA, IIIB, IIIC) were evaluated. Lip seal was recorded by observing the child's appearance in a resting position as they entered the room before they were aware of the pending examination. Incompetent lip seal was defined as the natural separation of the lips.[28,29] After the physical and oral examinations, orofacial functional evaluations were performed in the order delineated below. All participants were placed in the same room under the same lighting conditions and used the same chairs and desks.

Occlusal force testing

The occlusal force was measured using Dental Prescale II (GC Corporation, Tokyo, Japan) (Fig. 1A) and occlusal force analysis software (Bite Force Analyzer; GC Corporation).[30] The subjects were instructed to practice biting in the maximum intercuspal position 2 or 3 times before the measurement. The measurement was taken with the Frankfort horizontal plane parallel to the floor surface so that the occlusal state was as stable as possible. During the measurement, the examiner took care to avoid allowing the patient to slide or brux when the upper and lower teeth touched, and the occlusal state was recorded for approx. 3 s while biting down (Fig. 1B).[31]

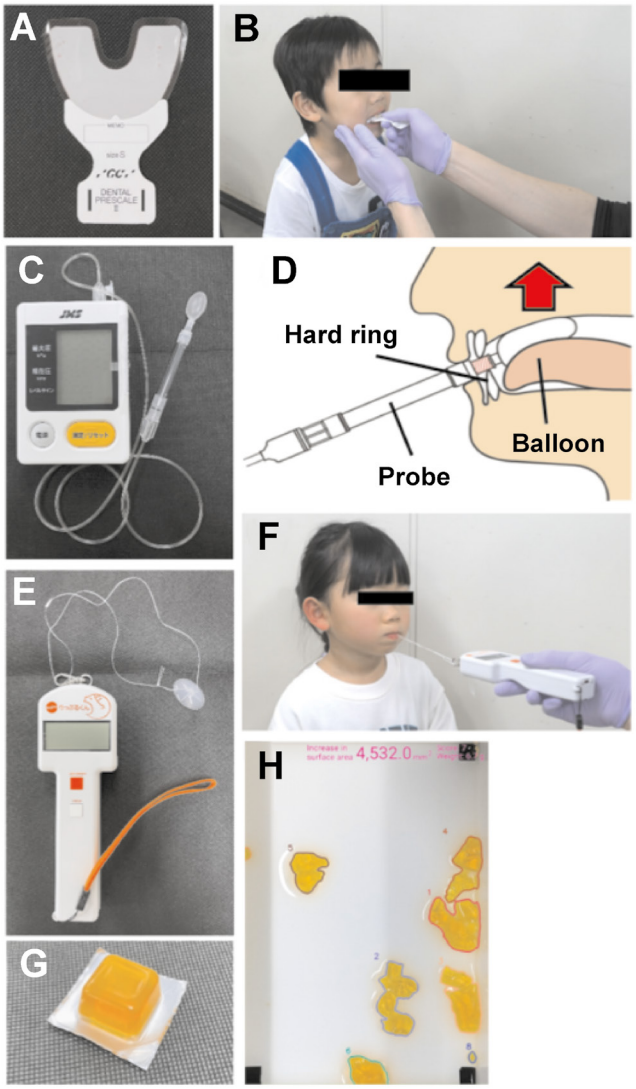


Fig. 1. Measurements of orofacial function performed in the present study

A. Dental Prescale II (GC Corporation, Tokyo, Japan) used for measuring occlusal force; B. Procedure for measuring occlusal force; C. Tongue pressure measurement device (JMS, Hiroshima, Japan); D. Intraoral positioning of the balloon during the measurement of maximum tongue pressure; E. Digital force gauge (Lipplekun; Shofu Inc., Kyoto, Japan) used for measuring lip-closing strength; F. Procedure for measuring lip-closing strength; G. Gummy jelly (20 mm × 20 mm × 10 mm; UHA Mikakuto, Tokyo, Japan) used for measuring masticatory performance; H. Gummy jelly particles after mastication.

Maximum tongue pressure

Maximum tongue pressure was measured with the use of a tongue pressure measurement device (JMS, Hiroshima, Japan) (Fig. 1C).[32] The subjects were instructed to practice biting the hard ring of the probe with their upper and lower incisors in an upright seated position and then raise their tongues and compress the balloon onto the palate, with the Frankfort plane maintained horizontally and the soles of their feet placed flat on the floor before the measurement (Fig. 1D). During the assessment, the examiner ensured that the balloon was positioned vertically between the palate and the tongue. The measurements were recorded 3 times with a rest interval between each, and the largest of the 3 measurements was used for analysis.

Lip-closing strength

Lip-closing strength was measured using a digital force gauge (Lipplekun; Shofu Inc., Kyoto, Japan) (Fig. 1E).[33] The subjects were instructed to insert a Lippule button (Shofu, Inc.) into the space between their incisors and lips, and to hold it with minimal mouth opening in a relaxed sitting position. Subsequently, they were asked to hold the button tightly in their mouths, and a string with a length of approx. 10 cm was tied to the center of the button. The strain force gauge was attached to the end of the string and pulled parallel to the floor. The strength, measured as the highest tension before the button was pulled from the mouth, was recorded 3 times with a rest interval between each, and the largest of the 3 measurements was used for analysis (Fig. 1F).

Masticatory performance

Masticatory performance was assessed using test gummy jellies (20 mm × 20 mm × 10 mm; UHA Mikakuto, Tokyo, Japan) (Fig. 1G).[34] The subjects were asked to sit in an upright position and chew the gummy jelly freely 30 times without swallowing, as they normally would. Thereafter, they were asked to spit out the particles onto cotton gauze placed over the top of a disposable cup without leaving any residue in their mouth. The gummy particles were then rinsed with tap water to prevent further dissolution and transferred into a prepared box containing 30 mL of water to prevent overlapping of the particles. Digital images of the box containing the comminuted pieces were captured using a smartphone camera and analyzed to calculate the increase in surface area and weight of the comminuted gummy jelly (Fig. 1H).[35]

Statistical analysis

Descriptive analyses included calculation of relative frequencies, mean ± standard deviation ($M \pm SD$) values, and medians (*Mes*) (interquartile ranges (*IQRs*)) in 2 groups

based on the SRS-2 total T scores: the high SRS-2 group (T scores ≥60); and the low SRS-2 group (T scores <60). The Shapiro–Wilk test was used to examine the normality of data distribution. The χ^2 test or Fisher's exact test, and Student's *t*-test or the Mann–Whitney *U* test, where appropriate, were used to compare the 2 groups. Additionally, Spearman's rank correlation coefficient was employed to investigate the relationships between the SRS-2 T score and each assessment item, as well as to evaluate the relationships between sleep disorder scores, dietary and meal habits, and orofacial functional scores. The data was analyzed using the IBM SPSS Statistics for Windows software, v. 28.0 (IBM Corp., Armonk, USA). The level of statistical significance was set at $p < 0.05$. For all significant comparisons, the effect sizes (Cohen's *d* or Cramer's *V*) or 95% confidence intervals (*CI*s) were reported. Additionally, power analysis was performed with the G* Power program, v. 3.1.9.6.[36] Given an anticipated effect size of 0.5, a two-tailed test, a significance level of 5%, and a total sample size of 56, the estimated power was 0.981.[37]

Results

Descriptive statistics

Of 56 children, 13 (23.2%) had SRS-2 T scores of 60 or more and were regarded as having signs of ASD (Table 1). The high SRS-2 group had substantially higher T scores than the low SRS-2 group ($p < 0.001$, Cohen's $d = -3.272$). There were no significant differences in age, sex, height, weight, or body mass index (BMI) between the 2 groups.

Sleep disorder scores

Table 2 presents sleep disorder scores according to the JSQ-ES. The high SRS-2 group showed significantly higher T scores for restless legs syndrome ($p < 0.01$; Cohen's $d = -0.981$), nighttime awakenings ($p < 0.01$; Cohen's $d = -1.078$), insomnia ($p < 0.05$; Cohen's $d = -0.679$), excessive daytime sleepiness ($p < 0.01$; Cohen's $d = -0.957$), daytime behavior ($p < 0.01$; Cohen's $d = -1.005$), irregular/delayed sleep phase ($p < 0.01$; Cohen's $d = -0.789$), and the sum of the 9 domains ($p < 0.001$; Cohen's $d = -1.125$) than the low SRS-2 group. There were no significant differences in SDB, morning symptoms, sleep habits, or sleep bruxism between the 2 groups.

Table 1. Descriptive characteristics of the study groups

Variable	High SRS-2 group (*n* = 13)	Low SRS-2 group (*n* = 43)	*p*-value
SRS-2 (T score)[a]	68.4 ±8.0	46.6 ±6.2	<0.001***
Age[a] [years]	7.6 ±0.9	7.9 ±0.9	0.287
Sex (male ratio)[b]	84.6	62.8	0.140
Height[a] [cm]	123.2 ±5.2	126.0 ±8.1	0.237
Weight[a] [kg]	25.0 ±4.2	26.3 ±5.4	0.431
BMI[a]	16.4 ±2.4	16.4 ±2.0	0.945

Data presented as mean ± standard deviation (*M* ±*SD*); [a] Student's *t*-test; [b] χ^2 test; *** statistically significant ($p < 0.001$); BMI – body mass index; SRS-2 – Social Responsiveness Scale, Second Edition.

Table 2. Sleep disorder scores in the study groups

Variable	High SRS-2 group (*n* = 13)	Low SRS-2 group (*n* = 43)	*p*-value
Restless legs syndrome (T score)	58.9 ±10.8	49.3 ±9.5	0.003**
Sleep-disordered breathing (T score)	59.5 ±11.2	54.3 ±8.7	0.082
Morning symptoms (T score)	53.4 ±9.2	50.1 ±9.6	0.267
Nighttime awakenings (T score)	57.4 ±12.1	47.2 ±8.6	0.001**
Insomnia (T score)	59.7 ±12.7	50.9 ±13.0	0.036*
Excessive daytime sleepiness (T score)	58.8 ±9.3	48.4 ±11.3	0.004**
Daytime behavior (T score)	58.6 ±8.4	48.5 ±10.4	0.002**
Sleep habits (T score)	54.5 ±10.8	54.4 ±10.8	0.992
Irregular/delayed sleep phase (T score)	57.3 ±9.6	48.0 ±12.4	0.009**
Sum of scores	63.0 ±10.0	50.6 ±11.3	<0.001***
Sleep bruxism (1–6)	3.9 ±1.9	3.0 ±1.8	0.102

Data presented as *M* ±*SD*. All items were analyzed with Student's *t*-test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Dietary and meal habits

The scores for dietary and meal habits are presented in Table 3. There were no significant differences between the 2 groups regarding the amount of food consumed per meal,

Table 3. Dietary and meal habits in the study groups

Variable		High SRS-2 group (*n* = 13)	Low SRS-2 group (*n* = 43)	*p*-value
Amount of food consumed per meal	excessive	15.4	18.6	0.791
	insufficient	7.7	2.3	0.361
	variable	38.5	37.2	0.935
Eating speed	too fast	38.5	16.3	0.088
	too slow	7.7	18.6	0.348
Picky eating	severe	23.1	18.6	0.158
	mild	61.5	37.2	–
	none	15.4	44.2	–
Behavior during meals	calm	30.8	76.7	0.002**
	standing up and playing	69.2	23.3	–

Data presented as percentage (%). All items were analyzed with χ^2 test; ** $p < 0.01$.

eating speed or picky eating. However, scores for standing up and playing during meals were significantly higher in the high SRS-2 group than in the low SRS-2 group ($p < 0.01$; Cramer's $V = 0.410$).

Orofacial functional scores

Results of the oral examination and the orofacial functional scores are displayed in Table 4. There were no significant differences between the 2 groups regarding the dmft/DMFT index, Hellman's dental age or incompetent lip seal. In the orofacial functional evaluation, owing to the gagging reflex, occlusal force could not be measured in 1 child in the high SRS-2 group, maximum tongue pressure could not be assessed in 2 children in the high SRS-2 group, and masticatory performance could not be evaluated in 1 child in the low SRS-2 group. There were no significant differences in the occlusal force, maximum tongue pressure or lip-closing strength between the 2 groups. However, masticatory performance was significantly lower in the high SRS-2 group than in the low SRS-2 group ($p < 0.05$; Cohen's $d = 0.773$).

Correlation of the SRS-2 scores with assessment items

Table 5 presents the correlation coefficients of the SRS-2 scores with orofacial functional and sleep assessment items. Regarding the sleep disorder items, restless legs syndrome ($r = 0.496$; 95% *CI*: 0.260–0.675, $p < 0.001$), SDB ($r = 0.393$; 95% *CI*: 0.137–0.600, $p < 0.01$), morning symptoms ($r = 0.310$; 95% *CI*: 0.044–0.536, $p < 0.05$), nighttime awakenings ($r = 0.557$; 95% *CI*: 0.338–0.719, $p < 0.001$), insomnia ($r = 0.461$; 95% *CI*: 0.218–0.651, $p < 0.001$), excessive daytime sleepiness ($r = 0.548$; 95% *CI*: 0.326–0.713, $p < 0.001$), daytime behavior ($r = 0.570$; 95% *CI*: 0.354–0.728, $p < 0.001$), irregular/delayed sleep phase ($r = 0.440$; 95% *CI*: 0.193–0.635, $p < 0.001$), the sum of sleep scores ($r = 0.569$; 95% *CI*: 0.353–0.727, $p < 0.001$), and sleep bruxism ($r = 0.425$; 95% *CI*: 0.175–0.624, $p < 0.01$) showed a significant correlation with the SRS-2 T score.

Additionally, in the orofacial functional items, occlusal force ($r = -0.325$; 95% *CI*: −0.549–−0.057, $p < 0.05$) and masticatory performance ($r = -0.293$; 95% *CI*: −0.524–−0.022, $p < 0.05$) were significantly negatively correlated with the SRS-2 T score (Fig. 2,3). With regard to dietary and meal habits, picky eating ($r = 0.324$; 95% *CI*: 0.058–−0.546, $p < 0.05$) and standing up and playing during meals ($r = 0.390$; 95% *CI*: 0.134–0.597, $p < 0.01$) were positively associated with the SRS-2 T score.

Table 4. Oral conditions and orofacial functional scores in the study groups

Variable		High SRS-2 group ($n = 13$)	Low SRS-2 group ($n = 43$)	*p*-value
dmft/DMFT index[a]		3.9 ±3.7	2.6 ±3.3	0.119
Hellman's dental age[b], *n*	IIA	0	2	0.587
	IIC	9	22	
	IIIA	1	10	
	IIIB	3	8	
	IIIC	0	1	
Incompetent lip seal[b] [%]		53.8	37.2	0.285
Occlusal force[a] [N]		445.0 ±239.9	570.4 ±265.0	0.145
Maximum tongue pressure[a] [kPa]		15.0 ±7.1	13.1 ±7.6	0.466
Lip-closing strength[a] [N]		6.8 ±2.1	7.5 ±2.1	0.292
Masticatory performance[a] [mm^2]		2,098.7 ±1,252.4	3,068.7 ±1,255.0	0.018*

[a] Student's *t*-test; [b] χ^2 test; * $p < 0.05$.

Table 5. Correlation between the Social Responsiveness Scale, Second Edition (SRS-2) scores and each assessment item

Assessment item	*r*	*p*-value
Age	−0.240	0.074
Sex (male ratio)	0.133	0.330
Height	−0.230	0.089
Weight	−0.137	0.313
BMI	0.034	0.802
Restless legs syndrome	0.496	<0.001***
Sleep-disordered breathing	0.393	0.003**
Morning symptoms	0.310	0.020*
Nighttime awakenings	0.557	<0.001***
Insomnia	0.461	<0.001***
Excessive daytime sleepiness	0.548	<0.001***
Daytime behavior	0.570	<0.001***
Sleep habits	0.070	0.608
Irregular/delayed sleep phase	0.440	<0.001***
Sum of JSQ-ES scores	0.569	<0.001***
Sleep bruxism	0.425	0.001**
Excessive food intake	0.078	0.568
Insufficient food intake	0.104	0.444
Variable food intake	0.193	0.154
Eating too fast	0.247	0.072
Eating too slowly	0.149	0.243
Picky eating	0.324	0.015*
Standing up and playing during meals	0.390	0.003**
dmft/DMFT index	0.209	0.122
Hellman's dental age	−0.157	0.249
Incompetent lip seal	0.074	0.587
Occlusal force	−0.325	0.015*
Maximum tongue pressure	−0.116	0.402
Lip-closing strength	−0.125	0.359
Masticatory performance	−0.293	0.030*

All items were analyzed using Spearman's rank correlation coefficient; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

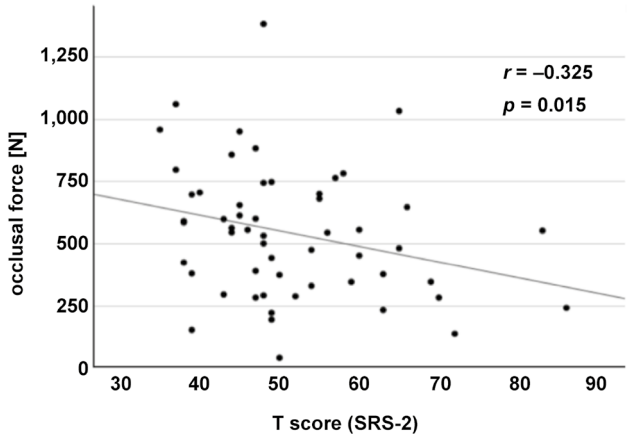


Fig. 2. Correlation between the T scores of the Social Responsiveness Scale, Second Edition (SRS-2), and the occlusal force

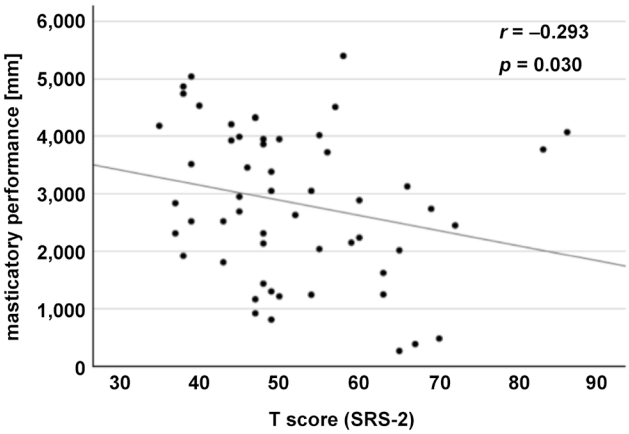


Fig. 3. Correlation between the T scores of the Social Responsiveness Scale, Second Edition (SRS-2), and masticatory performance

Correlation of dietary and meal habits and orofacial factors with sleep disorder scores

The correlation of dietary and meal habits and orofacial factors with sleep disorder scores is presented in Table 6. Excessive food intake was associated with SDB (r = 0.457; 95% *CI*: 0.212–0.647, p < 0.001), excessive daytime sleepiness (r = 0.307; 95% *CI*: 0.040–0.533, p < 0.05), irregular/delayed sleep phase (r = 0.311; 95% *CI*: 0.044–0.536, p < 0.05), the sum of JSQ-ES T scores (r = 0.304; 95% *CI*: 0.037–0.531, p < 0.05), and sleep bruxism (r = 0.276; 95% *CI*: 0.007–0.509, p < 0.05), while insufficient food intake was significantly correlated with SDB (r = –0.284; 95% CI: –0.515–-0.015, p < 0.05). Eating too fast showed significant associations with restless legs syndrome (r = 0.267; 95% *CI*: –0.003–0.501, p < 0.05), insomnia (r = 0.301; 95% *CI*: 0.033–0.528,

Table 6. Correlation of dietary habits and orofacial factors with sleep-related factors

Assessment item	Excessive food intake	Insufficient food intake	Variable food intake	Picky eating	Standing up and playing	Masticatory performance
Restless legs syndrome	–	–	0.267	0.289	0.301	–
	–	–	0.046*	0.031*	0.022*	–
Sleep-disordered breathing	0.457	–0.284	–	–	–	–
	<0.001***	0.034*	–	–	–	–
Morning symptoms	–	–	–	–	–	–
	–	–	–	–	–	–
Nighttime awakenings	–	–	–	–	–	–
	–	–	–	0.002**	–	–
Insomnia	–	–	0.301	–	–	–
	–	–	0.024*	–	–	–
Excessive daytime sleepiness	0.307	–	0.328	–	–	–
	0.021*	–	0.014*	–	–	–
Daytime behavior	–	–	–	0.395	–	–0.277
	–	–	–	0.003*	–	0.040*
Sleep habits	–	–	–	–	–	–
	–	–	–	–	–	–
Irregular/delayed sleep phase	0.311	–	–	0.301	–	–
	0.020*	–	–	0.024*	–	–
Sum of scores	0.304	–	–	0.326	–	–
	0.023*	–	–	0.014*	–	–
Sleep bruxism	0.276	–	0.284	–	0.273	–
	0.039*	–	0.034*	–	0.041*	–

Only significant items are listed. The upper rows present Spearman's rank correlation coefficients, while the lower rows display p-values; * p < 0.05; ** p < 0.01; *** p < 0.001.

$p < 0.05$), excessive daytime sleepiness ($r = 0.328$; 95% *CI*: 0.063–0.550, $p < 0.05$), and sleep bruxism ($r = 0.284$; 95% *CI*: 0.015–0.515, $p < 0.05$). Additionally, picky eating demonstrated correlations with restless legs syndrome ($r = 0.289$; 95% *CI*: 0.020–0.519, $p < 0.05$), nighttime awakenings ($r = 0.402$; 95% *CI*: 0.148–0.607, $p < 0.01$), daytime behavior ($r = 0.395$; 95% *CI*: 0.140–0.601, $p < 0.01$), irregular/delayed sleep phase ($r = 0.301$; 95% *CI*: 0.034–0.528, $p < 0.05$), and the sum of JSQ-ES T scores ($r = 0.326$; 95% *CI*: 0.061–0.548, $p < 0.05$), while standing up and playing during meals was related to restless legs syndrome ($r = 0.301$; 95% *CI*: 0.038–0.532, $p < 0.05$) and sleep bruxism ($r = 0.273$; 95% *CI*: 0.003–0.506, $p < 0.05$). Furthermore, masticatory performance was associated with daytime behavior ($r = -0.277$; 95% *CI*: −0.511–−0.005, $p < 0.05$).

Discussion

The results of our study reveal that children with signs of ASD have problems with orofacial function as well as with eating habits and sleep. Although healthy children who had not been diagnosed with neurodevelopmental disorders by a doctor participated in the present study, 23.2% of the participants had the SRS-2 T scores of 60 or more (Table 1). In the SRS-2, a T score of 60 or above is typically associated with clinically significant ASD.[24] The high SRS-2 group had a T score of 68.4 ±8.0, indicating a population with moderate ASD symptoms, while the low SRS-2 group had a T score of 46.6 ±6.2, reflecting a population without clinically significant ASD. These results show that the 2 groups differed significantly in the severity of ASD signs. High-functioning individuals with ASD are often diagnosed at a later age than recommended and may remain undiagnosed and latent.[3] Additionally, some of the participants in the present study were referred by general dentists to the Pediatric Dentistry Clinic due to uncooperative behavior during dental treatment. Therefore, it is likely that there was a certain amount of latent ASD reflected in the SRS-2 T scores of the participants. However, uncooperative behavior during dental treatment may be an indicator of developmental problems, including orofacial dysfunction and sleep disorders. In this regard, the generalizability of the present results to the typical pediatric population should be interpreted with caution.

The high SRS-2 group had significantly higher T scores than the low SRS-2 group for the majority of items related to sleep disorders (Table 2). The difference in the T scores between the high SRS-2 group (63.0 ±10.0) and the low SRS-2 group (50.6 ±11.3) can be regarded as clinically significant.[26] Most children with ASD have sleep problems, and their sleep is characterized by reduced total sleep time, longer sleep latency and decreased sleep efficiency,[5–7] which was reflected in the present results for nighttime awakenings, insomnia, excessive daytime sleepiness, daytime behavior, and irregular/delayed sleep phase, all of which were considered clinically significant.[26] Additionally, most scores for the sleep disorder items were significantly positively correlated with the SRS-2 T scores (Table 5), indicating that the severity of these sleep problems is associated with the severity of ASD symptoms, as found in a previous study.[9] Furthermore, the T scores for SDB were significantly positively correlated with the SRS-2 T scores (Table 5), which suggests that even among healthy children, those with signs of ASD tend to have a certain level of SDB. This association with signs of ASD may be evident in morning symptoms as well (Table 2,5).

Although the frequency of eating too fast and picky eating was higher in the high SRS-2 group compared to the low SRS-2 group, no significant differences were observed. However, standing up and playing during meals was reported significantly more frequently in the high SRS-2 group (Table 3). Additionally, picky eating and standing up and playing during meals were significantly and positively correlated with the SRS-2 T score (Table 5). Limited food preference is known to be the most common feeding behavior in children with ASD.[11] Our results suggest that the severity of ASD symptoms is associated with the severity of picky eating. Additionally, leaving the table is one of the most frequently demonstrated disruptive mealtime behaviors in children with feeding problems and can indicate food refusal.[38,39] In the present study, standing up and playing during meals may also be attributed to food refusal. The developmental stage from age 3 to 6 is fundamental to physiological changes in growth and adaptability, and habits established during this time can persist throughout an individual's entire life.[40] Children with ASD demonstrate sensory preferences and difficulty with motor control.[38,39] These sensitivities can be associated with a restricted intake of tolerated foods as well as low masticatory performance in the studied age group.

Few studies have investigated the relationship between neurodevelopment and orofacial function in children, and little quantitative data is available on oral function in children with ASD. The strength of the present study lies in the analysis of dynamic orofacial function (masticatory performance) and static orofacial function (lip pressure, tongue pressure) in early elementary school children with signs of ASD. The present study showed that masticatory performance was significantly lower in the high SRS-2 group than in the low SRS-2 group (Table 4). Although occlusal force, maximum tongue pressure and lip-closing strength were lower in the high SRS-2 group than in the low SRS-2 group, there were no significant differences in these values between the 2 groups, nor in the dmft/DMFT scores and the frequency of incompetent lip seal. Additionally, occlusal force and masticatory performance were significantly negatively correlated with the SRS-2 T score (Table 5). Although children with ASD frequently present with orofacial dysfunction characterized by excessive drooling and an open mouth at rest,[13] the condition is not necessarily the same, and the pathophysiology is not

uniform, as there is a great deal of individual variation due to differences in body size as well as in the tension and relaxation of each muscle. However, mastication, due to its complex coordinated movement involving the perioral and masticatory muscles, can represent a common problem with orofacial function among children with signs of ASD. Focusing on developmental coordination disorder, which occurs in approx. 80–90% of children with ASD,[41] may be useful for understanding the association between whole-body and orofacial movements in future research.

Orofacial conditions, including tonsillar hypertrophy, adenoid hypertrophy, retrognathic mandible, and lingual abnormalities, have been linked to sleep problems, especially OSA.[42,43] Additionally, recent studies have reported that dietary intake and breakfast habits are associated with sleep disorders in children.[44] In the present study, the amount of food consumed per meal, eating speed, picky eating, and standing up and playing during meals were significantly related to sleep problems, although these items were binary or categorical variables (Table 6). The amount of food consumed per meal and eating speed may be indirectly related to sleep problems through obesity,[45] and picky eating and eating habits may be indirectly associated with sleep disorders through neurodevelopment. However, considering that chewing ability is also related to sleep disorders (Table 6), eating problems may be an indicator of oral function, which is closely related to sleep and neurodevelopment.[46] Furthermore, sleep problems in children can be negatively correlated with oral function and oral hygiene[47,48]; thus, further research is needed to investigate the relationships between oral health, sleep health and neurodevelopment.

Limitations

The present study had some limitations that should be considered. First, this cross-sectional study analyzed data from early elementary school children aged 6–9 years, with a relatively small sample size. To mitigate this limitation, simplified measurements were employed to evaluate orofacial function. These multiple measurements can be challenging in uncooperative subjects. The results were based on exact and objective data with only a few dropouts; however, sample bias (e.g., the study population was not representative of the general population and the study did not employ a strictly controlled design) could not be avoided, and the generalizability to the typical pediatric population should be interpreted with caution. Additionally, the multiple measurements may have caused fatigue in the children, and the values should be analyzed considering this factor. Based on the results of this study, future cohort and comparative studies are warranted to elucidate the relationship between oral function and sleep in children with developmental difficulties. Second, all factors related to sleep disorders and signs of ASD in the present study were evaluated based exclusively on scaled quantitative assessment questionnaires, and these judgments were not definitive. However, subjective assessments using questionnaires may be more closely related to specific clinical symptoms than objective sleep assessments such as polysomnography.[49] Additionally, it is recommended that routine sleep screening be incorporated into dental clinical practice because there could be a specific subgroup of SDB patients that may go undetected in general pediatric clinics.[23] The screening for ASD with the SRS-2 in this study has not been validated against clinical observational diagnoses such as the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2),[3] and participants may have been misclassified as having other disorders that cause similar symptoms. Nevertheless, questionnaire-based assessment remains the only strategy for the screening of sleep disorders and signs of ASD in a pediatric dentistry clinic, and it is a common strategy, especially for early elementary school children. Signs of ASD and sleep disorders are considered to have been assessed adequately in the present study. Third, although we demonstrated relationships between signs of ASD and orofacial function as well as sleep disorders, these connections do not indicate causation but merely correlation. Hypotonia, impaired coordination, and the resulting mouth breathing and maxillofacial underdevelopment, which are characteristic of children with ASD, may be associated with orofacial dysfunction and sleep problems.[14,50] A detailed analysis that considers individual factors such as age and socioeconomic factors will likely clarify the underlying mechanisms. Fourth, multiple regression analyses could not be conducted, and some factors and biases could not be considered due to the small sample size. Additionally, many statistical tests in our study were performed as exploratory analyses, and the statistical significance level was not adjusted. Therefore, insufficient power and statistical errors may exist, and the results should be interpreted with caution. Nevertheless, the present study quantified dynamic orofacial function as well as static orofacial function in early elementary school children, revealing that children with signs of ASD have low masticatory performance, a type of dynamic oral function, and indicated the importance of focusing on functional aspects as well as behavioral, psychological and nutritional aspects in ASD-related eating problems. Further studies focusing on orofacial and neurodevelopmental conditions would be useful for assessing vital functions in children with and without signs of ASD.

Conclusions

Within the limitations of this experimental study, it can be concluded that low masticatory performance, as well as behavioral and psychological factors, may contribute to feeding issues in early elementary school children with signs of ASD. Additionally, the severity of ASD symptoms is associated with the severity of problems with orofacial

function, feeding behavior and sleep. The results of this study underscore the importance of interventions targeting orofacial function and sleep disturbances in children with signs of ASD.

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the Graduate School of Dentistry at the University of Osaka, Japan (approval No. R3-E5). Written informed consent for study participation was obtained from all parents/guardians, and verbal agreement and written informed assent were obtained from all children.

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

Effectiveness of heartfulness meditation in the management of recurrent aphthous ulcers

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Abstract

Background. Recurrent aphthous ulcers (RAUs) are the most prevalent form of painful oral mucosal disorder. Conventional treatment for RAUs involves the administration of tetracycline, which has been shown to alleviate clinical signs and symptoms. However, this medication has been associated with a number of adverse effects. Heartfulness meditation (HM) has been shown to facilitate natural healing of ulcers without complications by developing a compassionate connection with oneself.

Objectives. The aim of the study was to evaluate the effectiveness of HM in the management of RAUs and to compare its effects with those of conventional treatment.

Material and methods. A parallel study was conducted among 54 patients affected with RAUs. The patients were divided into 3 groups: group 1 – HM; group 2 – tetracycline treatment; and group 3 – combination of tetracycline treatment and HM. The evaluated outcomes included pain levels and the duration of ulceration. One-way analysis of variance (ANOVA) and paired *t*-test were applied to identify significant differences between the groups.

Results. A substantial decrease in pain intensity was observed during HM. The group that engaged in meditation alongside tetracycline treatment exhibited the lowest pain scores. At follow-up intervals of 3, 5 and 7 days, pain levels between the tetracycline group and the HM group were similar. The group that received a combination of tetracycline treatment and HM demonstrated the shortest duration of ulcers (7.167 ±0.383 days).

Conclusions. The study concluded that when HM was practiced in addition to tetracycline treatment, it demonstrated superior efficacy in reducing pain at the shortest time. Furthermore, the study noted a reduction in the duration of ulcers when HM was used in conjunction with tetracycline, as compared to the practice of HM alone.

Keywords: pain, stress, meditation, tetracycline, oral mucosal ulcers

Highlights

- The study demonstrates that heartfulness meditation complements conventional treatments for recurrent aphthous ulcers.
- Combining heartfulness meditation with tetracycline accelerates healing and reduces pain more effectively than tetracycline alone.
- At follow-up, heartfulness meditation alone reduces pain similarly to tetracycline, highlighting its potential as a non-pharmacological approach.
- Findings suggest integrating heartfulness meditation with standard therapy to enhance treatment outcomes for recurrent aphthous ulcers.

Introduction

Recurrent aphthous ulcers (RAUs), also known as canker sores, are the most prevalent form of painful oral mucosal disorder.[1] Recurrent aphthous ulcers manifest during infancy or adolescence as multiple, tiny ulcers with round or ovoid shapes, constricted edges, yellow or grey floors, and erythematous haloes.[2] The etiology of RAUs is multifactorial, involving stress, dietary influences, nutritional deficiencies, and immune system dysfunction.[3,4]

The preferred treatment for RAUs is tetracycline. While tetracycline has been shown to reduce inflammation and promote healing of the ulcers, it does not address their underlying cause.[5] The overuse of antimicrobials leads to the development of antibiotic-resistant bacteria, which can complicate treatment of future infections.[6] In addition, tetracycline can have side effects such as upset stomach and discoloration of teeth, which can limit its use. Lifestyle changes have been demonstrated to be effective in the prevention of the recurrence of RAUs.[7]

Heartfulness meditation (HM) is a form of meditation that focuses on developing compassion, inner peace and a deeper connection with oneself and others. The practice of meditation can effectively reduce stress and promote relaxation, supporting the body's natural healing processes.[8] While scientific literature on the effects of HM on RAUs remains limited, some studies have shown that meditation may have a positive influence on overall physical and mental health. For instance, a study by Taub et al. demonstrated that mindfulness-based stress reduction (MBSR) decreased symptoms of depression and anxiety in cases with chronic illness.[9]

Studies identify a definite relationship between RAUs and stress. In fact, stress is considered a triggering factor for the development of ulcers. It can affect and impair the regular functioning of the immune system. This, in turn, weakens the body's defense mechanisms, making it more susceptible to infections and inflammatory diseases such as RAUs. Stress can lead to hormonal imbalances, specifically by increasing the levels of stress hormones such as cortisol. These hormonal changes may affect various physiological processes, including the healing and repair mechanisms of the oral mucosa, potentially contributing to the development of ulcers. Given the established role of stress as a triggering factor for RAUs, the potential benefits of HM, a practice aimed at stress reduction and relaxation, are a compelling area for investigation.[10] Hence, the present study was conducted to answer the following research question: "Is the practice of HM as effective as conventional treatment for managing RAUs?".

Material and methods

Study setting

The present double-blind, three-arm clinical trial was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Ethics Committee of the Department of Public Health Dentistry, People's College of Dental Sciences & Research Centre, Bhopal, India (IEC No. EC 202133). After explanation of the risks and benefits of the study, all patients and their parents provided written consent to participate in the study.

Participants

The study population comprised healthy individuals of both sexes, aged 18–45 years, who presented with ulcers persisting for more than 24 h. Patients with systemic conditions, allergy to tetracyclines, and those undergoing any other treatment for RAUs, as well as pregnant and lactating women were excluded. The selection process is depicted in Fig. 1.

The assessment of aphthous ulcers was based on a review of patient's medical history and clinical examination. The clinical examination criteria included the following: the presence of ulcers on non-keratinized oral mucosa (i.e., the inside of the lips, cheeks, floor of the mouth, or underside of the tongue); round or oval-shaped with well-defined borders; typically manifesting as a shallow surface with a depressed center surrounded by an inflamed and slightly elevated border; causing pain and discomfort, especially during activities such as eating, drinking, or speaking. The clinical examination was conducted by a single examiner to ensure reproducibility.

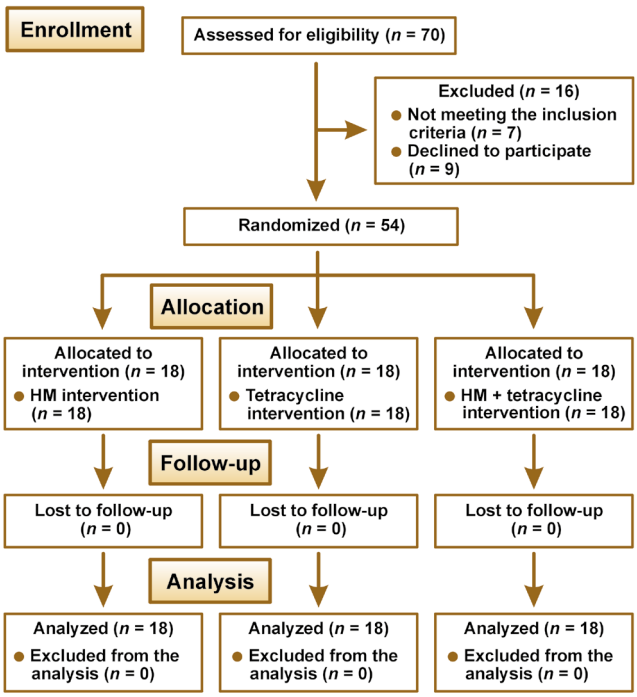


Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) diagram of the study

HM – heartfulness meditation.

Sample size

The sample size was calculated based on the following formula (Equation 1):

$$N = 2 \times \left(\frac{Z_{1-\alpha/2} + Z_{1-\beta}}{\delta} \right)^2 \times S^2 \quad (1)$$

where:

N – size per group;

δ – effect size or a clinically acceptable margin (as per Zeeshan et al.[11]);

$Z_{1-\alpha/2}$ – critical value from the standard normal distribution for a given level of significance (α);

$Z_{1-\beta}$ – critical value for the study's statistical power ($1-\beta$).

S^2 – pooled standard deviation of both comparison groups.

The power was conventionally set at 0.80.

Considering a 10% loss to follow-up, the final sample was rounded off to 17.6, thereby establishing 18 subjects in each group. The final sample size was determined to be 54.

Randomization and blinding

The patients were randomly assigned to one of the 3 groups based on computer-generated random numbers. To ensure the blinding of the study participants, patients from different groups were recalled on separate days so that they never met.

Groups and intervention

The study population was comprised of 3 groups; group 1 consisted of 18 subjects who underwent the HM program; group 2 consisted of 18 individuals who were treated with tetracycline; and group 3 included 18 patients who received a combination of both interventions.

Group 1 participants underwent an orientation session, during which they received instruction on the meditation procedure. The presentation, delivered via PowerPoint, encompassed the benefits of meditation, heartfulness practice, rejuvenation technique, and bedtime prayer meditation. The presentation was also shared with the participants to reinforce the intervention. The HM practice entailed a sequence of 5–7 min of relaxation, followed by 20 min of meditation. The participants were instructed to close their eyes and focus on a source of light within their hearts. The evening session included 15 min of rejuvenation, during which the patients were guided to visualize stress and heaviness leaving their bodies in the form of smoke or vapor. A bedtime prayer meditation was recommended for introspection of the day for 5 min before going to bed. All patients were provided with a practice tracker sheet and were requested to document their session attendance.

In group 2, the first application of tetracycline was conducted in the clinic to all participants to demonstrate the method of application. The medication was administered in the form of a capsule, which was to be mixed with a small amount of saline and applied to the ulcer thrice daily.

Group 3 received a combination of tetracycline and HM practice.

Outcomes assessed

The assessed outcomes included the severity of pain and the duration of ulceration. Pain was evaluated using the visual analogue scale (VAS) on the 1st, 3rd, 5th, and 7th day. The duration of ulceration was recorded in days.

Statistical analysis

The results were analyzed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). One-way analysis of variance (ANOVA) was applied to identify statistically significant differences between the groups at different time intervals. A paired *t*-test was conducted to ascertain the presence of any variation in pain and ulcer duration on the 1st and 7th day in each group. This analysis was conducted at a 5% level of significance.

Results

A total of 54 RAU patients were examined. The mean age of the participants was 27.59 ±5.31 years.

The predominance of female patients was noted, with 37 (68.5%) cases being assessed. When the intensity of pain was evaluated at various time intervals, no significant differences were observed between the groups on the 1st day (p = 0.699). On the 3rd day, the pain scores in groups 1 and 2 were comparable, with means of 5.167 ±0.618 and 5.000 ±0.767, respectively. However, group 3 exhibited a significantly lower pain score (3.944 ±0.802; p = 0.000). Similar findings were noted on the 5th and 7th day (Table 1).

Table 1. Comparison of pain intensity at various time intervals between the groups

Day	Group	VAS *M* ±*SD*	*F* statistic	*p*-value
1st day	group 1	6.222 ±0.647	0.361	0.699
	group 2	6.000 ±0.907		
	group 3	6.056 ±0.873		
3rd day	group 1	5.167 ±0.618	14.694	0.000*
	group 2	5.000 ±0.767		
	group 3	3.944 ±0.802		
5th day	group 1	3.889 ±0.758	11.103	0.000*
	group 2	3.833 ±0.618		
	group 3	2.944 ±0.639		
7th day	group 1	1.667 ±0.485	4.071	0.023*
	group 2	1.722 ±0.575		
	group 3	1.278 ±0.461		

* statistically significant (p < 0.05, ANOVA); VAS – visual analogue scale; *M* – mean; *SD* – standard deviation.

Within-group comparisons revealed a gradual reduction in pain scores across all groups from the 1st day to the 7th day. The findings obtained on the 3rd day were more pronounced for group 3 as compared to groups 1 and 2 (Table 2). This finding indicates that HM reduced pain intensity at the shortest time interval. The pain levels in the tetracycline group and the HM group were comparable at all follow-up time points.

The healing process for ulcers was the longest in group 2 (8.056 ±0.639 days), while the condition was cured the fastest in group 3 (7.167 ±0.383 days). This discrepancy was found to be statistically significant (p = 0.002) (Table 3). The findings indicate that the combination of tetracycline and HT resulted in the lowest pain scores and the most rapid healing rate.

Table 3. Comparison of the duration of ulceration between the groups

Groups	Duration of ulceration [days] *M* ±*SD*	*F* statistic	*p*-value
Group 1	7.722 ±0.752	9.714	0.002*
Group 2	8.056 ±0.639		
Group 3	7.167 ±0.383		

* statistically significant (p < 0.05, ANOVA).

Discussion

The current study revealed that conventional treatment with tetracycline in combination with HM yielded optimal outcomes in terms of pain reduction and ulcer healing. This study is pioneering in its field, as there have been no previous studies that have examined this particular aspect. The practice of yoga and meditation, with their emphasis on stress reduction and pain management, have been integral components of therapeutic interventions in learning schools and settings since ancient times. Numerous researchers have utilized the methods of contemporary brain research to examine the effects of meditation on the mind and body.

The exact cause of RAUs remains unknown. However, current hypotheses attribute the occurence of RAUs to a combination of genetic, environmental and immunological factors. The mechanism underlying the pain experienced in RAUs is not fully understood, but several theories have been postulated to explain this phenomenon. One such theory suggests that the pain is caused by the release of inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β), in response to tissue damage. These cytokines have been observed to activate pain receptors, including nociceptors, which send pain signals to the brain. Another theory stipulates that the pain is caused by the activation of the immune system in response to the ulcer. The immune system releases inflammatory mediators, such as prostaglandins and leukotrienes, which can also activate pain receptors. Additionally, pain may be caused by nerve damage or irritation, as the ulcers can damage nerve endings in the affected area.[12] Overall, the exact pain mechanism in RAUs is not fully understood and may involve a combination of factors. Treatment regimens for RAUs are aimed at relieving pain and promoting ulcer healing.

Table 2. Within-group comparison of pain intensity at various time intervals

Group	VAS *M* ±*SD*				*F* statistic	*p*-value
	1st day	3rd day	5th day	7th day		
Group 1	6.222 ±0.647	5.167 ±0.618	3.889 ±0.758	1.667 ±0.485	171.805	0.000*
Group 2	6.000 ±0.907	5.000 ±0.767	3.833 ±0.618	1.722 ±0.575	114.554	0.000*
Group 3	6.056 ±0.873	3.944 ±0.802	2.944 ±0.639	1.278 ±0.461	141.703	0.000*

* statistically significant (p < 0.05, ANOVA).

Studies have demonstrated that RAUs are triggered by psychological stress.[13–15] A possible explanation for the efficacy of HM in RAU management is its capacity to reduce stress. Research indicates that HM can reduce stress by activating the parasympathetic nervous system, which is responsible for the "rest and digest" response. This response leads to a decrease in heart rate, blood pressure, and other physiological responses to stress. Furthermore, HM has been shown to reduce amygdala activity, thereby mitigating feelings of anxiety and stress. The amygdala, a key component of the brain, plays a pivotal role in processing fear and other negative emotions. Heartfulness meditation has also been shown to enhance the activity of the prefrontal cortex, which is responsible for executive functions such as decision-making, problem-solving and self-control. Thus, HM can help individuals to better manage their emotions and respond to stressful situations in a more effective manner.[16]

Heartfulness meditation has shown beneficial effects on various aspects of health. Gurram et al. assessed anxiety levels in 26 individuals undergoing impacted third molar surgery who practiced HM.[17] They observed that a single session of HM effectively reduced intraoperative anxiety levels in a target population.[17] Khan et al. assessed the efficacy of Raja yoga meditation and pranayama in patients with myofascial pain dysfunction syndrome (MPDS) and compared the results with continued traditional non-invasive therapy techniques.[18] Pain and inflammation improved significantly in individuals who practiced meditation. The therapeutic intervention had a prolonged effect, accompanied by a reduction in anxiety and stress. The authors concluded that Raja yoga meditation and pranayama, combined with conventional non-invasive intervention, showed promising results in MPDS cases when compared to either modality alone.[18] Thimmapuram et al. determined the variation in all domains of health-related quality of life (HQoL) among 3,164 practitioners of yoga and meditation across 39 countries.[19] It was noted that individuals with more than 2 years of meditation experience had better outcomes for HQoL as compared to those who did not practice or had less than 1 year of experience.[19]

Research has demonstrated the effectiveness of meditation on chronic mucosal and skin conditions associated with stress. In a clinical trial conducted on 57 participants, González-Moret et al. found that inflammatory biomarkers were enhanced in patients with inflammatory bowel disease (IBD) when treated with mindfulness-based therapy.[20] Inflammatory bowel disease is frequently associated with psychological distress, and thus stress management is the recommended line of therapy. Concurrently, Meesters et al. reported enhanced wound healing in 23 individuals who practiced meditation.[21] In addition, a reduction in cytokines (IL-8 and placental growth factor) was observed among these individuals. Taub et al. expressed promising interventions for systemic lupus erythematosus patients, encompassing outcomes such as quality of life, the psychological impact of the condition, and shame.[9] These studies suggest that an intervention capable of effectively reducing stress can alleviate the distress experienced by RAU patients. The validity of VAS as a pain assessment scale has been widely studied, and it is considered a reliable and valid tool for quantifying pain intensity.[22]

Limitations

The present study is subject to certain limitations. Longitudinal research is necessary to establish the causality between meditation and the outcomes of the study. Also, the possibility of inclusion bias cannot be dismissed, as the study's participants were voluntarily enrolled. The influence of dietary habits, oral hygiene practices and medication changes was not considered in the present study. These factors, if present, could have confounded the results, thereby complicating the isolation of the intervention effect.

Conclusions

The severity of pain was found to be reduced in the group that practiced HM along with conventional treatment. The findings suggest a potential impact of HM on brain activity, which may contribute to disrupting the cycle of pain. Further studies are necessary to determine the specific effects of HM on pain in RAUs. However, incorporating meditation into one's daily routine has the potential to provide overall benefits for physical and mental health. It is therefore strongly recommended that HM be integrated with pharmacological interventions to ensure optimal results.

Ethics approval and consent to participate

The study was approved by the Institutional Ethics Committee of the Department of Public Health Dentistry, People's College of Dental Sciences & Research Centre, Bhopal, India (IEC No. EC 202133). All patients and their parents provided written consent to participate in 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.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Efficacy of silymarin on calprotectin and S100A12/EN-RAGE in chemotherapy-induced oral mucositis

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Abstract

Background. Oral mucositis (OM) is an oral complication of chemotherapy with agents such as 5-fluorouracil (5-FU). Silymarin (SM) has been utilized therapeutically as a cytoprotective medication.

Objectives. The aim of the study was to evaluate the efficacy of SM as a mucoprotective agent in the treatment of chemotherapy-induced oral mucositis (CIOM) induced by 5-FU, and to assess calprotectin (CP) and S100A12/EN-RAGE levels as novel biomarkers for the evaluation of mucositis.

Material and methods. Fifty Wistar albino rats of both sexes were divided randomly into 5 groups. Three groups were treated with different doses of SM (2 mg/kg, 4 mg/kg, 6 mg/kg) for 10 days. Subsequently, the animals from groups I–III received intraperitoneal injections of 5-FU (60 mg/kg) on days 11–14. The 4th group was treated with dimethyl sulfoxide (DMSO) as a control for 10 days, followed by 2 doses of 5-FU, and the 5th group served as a second control and received 5-FU only. The buccal mucosa of the animals was gently injured with a 0.8-mm orthodontic wire (2×6 strokes) under anesthesia. The efficacy of SM treatment on CP and S100A12/EN-RAGE levels in CIOM was evaluated. Macroscopic and histopathological findings were analyzed, and the correlation coefficients between the studied parameters were calculated.

Results. The study demonstrated a significant protective effect of SM in chemotherapy-induced buccal mucositis in a dose-dependent manner. Mucositis ulceration was not observed, in contrast to severe OM lesions noted in the control groups. Analysis using a 95% confidence interval (*CI*) showed highly significant differences between the groups regarding the levels of CP and S100A12/EN-RAGE, as well as very strong correlations with OM ulcers.

Conclusions. Based on the macroscopic and histopathological appearance, the study confirmed the dose-dependent protective efficacy of SM treatment in CIOM. In addition, the findings suggest a novel mucoprotective mechanism of SM mediated through its effects on CP and S100A12/EN-RAGE levels. A very strong positive correlation was found between plasma CP and S100A12/EN-RAGE levels and the presence of OM ulcers, suggesting that these biomarkers may be useful in the clinical assessment of CIOM.

Keywords: calprotectin, chemotherapy-induced oral mucositis, S100A12/EN-RAGE, silymarin

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Highlights

- The study evaluates the efficacy of silymarin in an animal model of chemotherapy-induced oral mucositis (CIOM).
- Silymarin reduces calprotectin and S100A12/EN-RAGE levels, demonstrating dose-dependent mucoprotective activity against CIOM.
- Plasma S100A12/EN-RAGE levels increase in untreated animals but decrease significantly after the administration of the highest silymarin dose, demonstrating dose-dependent mucoprotection against CIOM.
- Macroscopic and histopathological findings further confirm dose-dependent protection of oral tissues by silymarin against CIOM.

Introduction

Oral mucositis (OM) is an inflammatory condition of the oral and oropharyngeal mucosa, often resulting from radiotherapy or chemotherapy.[1] Preventive strategies for OM are important, as the condition can decrease the effectiveness of chemotherapy.[2,3] Chemotherapy can cause OM, which typically develops within 5–14 days after treatment.[4] It can initially present as erythema of the oral mucosa and then progress to ulcers. These ulcers are usually located on the non-keratinized surfaces of the oral cavity, such as the buccal mucosa.[5]

5-fluorouracil (5-FU) is a major cause of OM because its administration may necessitate dose reduction, treatment delay or discontinuation of chemotherapy, which occurs in more than 15% of cases receiving 5-FU.[6] 5-fluorouracil is a promising pyrimidine analogue that largely interrupts DNA and RNA synthesis through its incorporation into DNA, thereby inhibiting mitosis and stimulating cell death in the dividing cells.[7] Additionally, 5-FU is the most commonly used chemotherapy agent for the treatment of various types of cancer. The interactions between the drug and cancer cells can be influenced by non-coding RNAs involved in the treatment process.[8] However, a major adverse effect of 5-FU is OM.[9] The intraperitoneal administration of 5-FU combined with superficial mechanical mucosal infusions resulted in ulcerative OM in animals.[10]

Calprotectin (CP) can induce the production of the provocative cytokines in the macrophages and monocytes throughout the activation of the p38 mitogen-activated protein kinase (MAPK) and nuclear factor κB (NF-κB) pathways.[11] Numerous studies have demonstrated that S100P contribute to leukocyte migration and adhesion. Furthermore, the release of S100A8/A9 promotes the migration of neutrophils and monocytes.[12] Studies have shown that CP can act as a toll-like receptor 4 (TLR4) agonist.[13]

The S100 protein family is a group of calcium-binding proteins that play a role in the regulation of inflammation and immune homeostasis. When activated by the receptor for advanced glycation end-products (RAGE), these proteins can trigger the production of pro-inflammatory cytokines.[14] S100A12 is overexpressed in inflammatory tissues, and serum S100A12 levels are elevated in numerous neurodegenerative, inflammatory, neoplastic, and metabolic disorders. As a result, the interactions between S100A12 and RAGE or soluble RAGE (sRAGE) receptors are thought to play an important role in disease pathogenesis.[15] During the initial phase of inflammation, modified proteins, pro-inflammatory cytokines and free radicals are released from cells within the oral mucosa, including tumor necrosis factor (TNF), interleukin (IL)-1β and prostaglandins. These mediators can cause further damage either directly or indirectly by enhancing vascular permeability, thereby increasing the absorption of cytotoxic drugs into the oral mucosa.[16] S100A12 activates the NF-κB signaling pathway, resulting in the upregulated expression of cytokines and other pro-inflammatory factors, such as IL-1β or TNF-α.[17]

Previous studies investigating the treatment of OM have evaluated the efficacy of dexamethasone, sucralfate mouthwash and olive leaf extract. However, no treatment has provided complete protection against OM.[18] The protective effects of silymarin (SM) on mucous membranes could be beneficial in the treatment of patients with OM caused by radiotherapy or chemotherapy.[19]

Silymarin is derived from *Silybum marianum* (milk thistle). It has been utilized therapeutically for many years as a hepatoprotective and cytoprotective agent. Pharmacological evidence shows that SM has a direct influence on cell-managing instruments, characteristics of reactive oxygen species (ROS) and anti-inflammatory activities. These properties may reduce the harmful effects of various drugs, such as cisplatin and amiodarone.[20] Additionally, SM acts as an anticancer, immunomodulatory, antiviral, and cardioprotective agent.[21,22]

Dimethyl sulfoxide (DMSO) is one of the most widely used pharmaceutical drugs in the life sciences industry. It is a universal solvent capable of dissolving both non-polar and polar compounds. Numerous studies have investigated the properties of DMSO in the treatment of various diseases.[23]

Material and methods

Reagents

The following reagents were used in the study: 5-FU, 50 mg/mL (Sigma-Aldrich, St. Louis, USA); SM, 87% powder (Sigma-Aldrich); 40% DMSO (Laboratory Rasayan, Anand, India); 95% diethyl ether (Alpha Chemicals, Panvel, India); 37–40% formaldehyde (AZ chem, Berlin, Germany); rat CP enzyme-linked immunosorbent assay (ELISA) kit (AZ chem); rat S100A12 ELISA kit (AZ chem).

Study design

The present study was designed to assess the efficacy and dose-dependent effects of various doses of SM as a mucoprotective agent against chemotherapy-induced oral mucositis (CIOM). Outcome measures included macroscopic evaluation, ulcer size measurement, histopathological examination, and the assessment of CP and S100A12/EN-RAGE levels. In addition, correlation analyses were performed between CP and S100A12/EN-RAGE levels, ulcer size, the methods of their reduction or prevention, and the other studied parameters.

Rats and housing

Adult Wistar albino rats of both sexes, aged more than 2 months and weighing 250–350 g, were obtained from the Faculty of Pharmacy at Isra University, Amman, Jordan. The animals were housed under standard laboratory conditions in polycarbonate cages containing a wood-shaving layer. Four rats were housed per cage. The animals were maintained at 20–25°C, with a relative humidity, under a 12-h light/12-h dark cycle. All rats were identified by tail markings, weighed and randomly allocated into 5 groups using Microsoft Excel (Microsoft Corp., Redmond, Washington). The study protocol was approved by the Institutional Committee for Ethics in Animal Use at the Faculty of Pharmacy, Isra University (registration No. 3-24/2019), and was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental design and study groups

Wistar albino rats of both sexes were randomly assigned to 5 groups (n = 10/group). The first 3 groups received intraperitoneal injections of SM at doses of 2 mg/kg, 4 mg/kg and 6 mg/kg, respectively. Silymarin was dissolved in DMSO and administered in a total injection volume of 1 mL once daily for 10 consecutive days. Beginning on day 11, all 3 groups received 5-FU (60 mg/kg) once daily for 4 consecutive days (days 11–14). The 4th group received DMSO (1 mg/kg) for 10 days, followed by the intraperitoneal administration of 2 doses of 5-FU. The 5th group received 5-FU (60 mg/kg) only and served as the positive control for the induction of OM. The animals were anesthetized with diethyl ether, after which their left cheeks were exposed and gently injured using a 0.8-mm steel orthodontic wire (2×6 strokes). The rats were weighed weekly to adjust the administered dose and to monitor their general health. The flowchart of the study design is presented in Fig. 1.

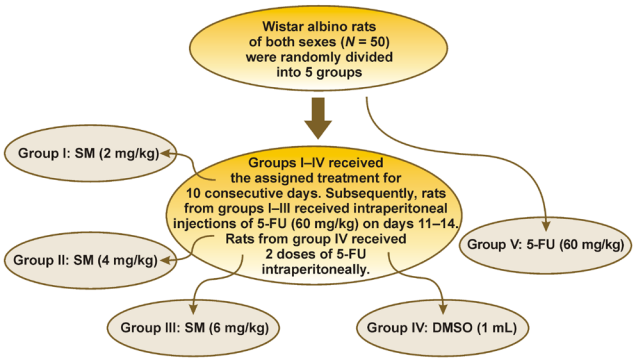


Fig. 1. Flowchart of the experimental design

SM – silymarin; 5-FU – 5-fluorouracil; DMSO – dimethyl sulfoxide.

Preparation of the dosage and samples

Silymarin and 5-FU solutions were prepared daily under sterile conditions immediately before administration. The administered dose was adjusted according to each animal's body weight. The rats were anesthetized with diethyl ether for approx. 5 min until loss of consciousness was achieved. Blood samples were collected from the optical vein using capillary tubes. The blood samples were immediately centrifuged at 5,200 rpm for 10 min, and the plasma was stored at –20°C until analysis.

The sample size was estimated using the G*Power software (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). Based on the calculated effect size, the minimum required sample size was 6 animals per group. To compensate for potential animal losses, the sample size was increased to 10 rats per group. Effect sizes were estimated using the mean values and standard deviations of the studies variables.[24]

Allocation and blinding

The animals were assigned to experimental groups using a computer-generated randomization sequence. Histopathological sections were independently evaluated by 2 blinded observers. The scores assigned for each section were then averaged and used for subsequent analyses.[25]

Histopathological study

The buccal mucosa and tongue tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at a thickness of 4 μm.[26] Sequential units were stained with hematoxylin and eosin (H&E) for general histological evaluation and Masson's trichrome stain for the assessment of collagen deposition. Histological sections were examined using a microscope (Labophot-2; Nikon, Tokyo, Japan) and a camera (OptikamB9; Optika S.R.L, Ponteranica, Italy). The tongues were stained with 1% toluidine blue prepared in 10% acetic acid for 1 min and subsequently rinsed several times with acetic acid to identify ulcerative and erosive lesions. Ulcer size and epithelial thickness of the mucosa were measured.

Assessment of calprotectin and S100A12/EN-RAGE by ELISA

Plasma CP and S100A12/EN-RAGE levels were determined using commercial ELISA kits according to the manufacturers' instructions.

Correlation analyses

Correlation analyses were performed between CP and S100A12/EN-RAGE levels and the studied parameters, including ulcer size and histopathological findings.

Statistical analysis

Data was analyzed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). Analysis of variance (ANOVA) was used to compare differences among the experimental groups. Pearson's correlation coefficient was calculated to evaluate associations between biomarker levels and the studied parameters. When ANOVA demonstrated significant differences, Duncan's test was used. Data was presented as the mean with the 95% confidence interval (*CI*). A *p*-value <0.05 was considered statistically significant. The G*Power 3.1.9.4. software was used to calculate the effect size and estimate the required sample size.

Results

A total of 50 Wistar albino rats, aged 8 weeks and weighing 250–350 g, were included in the study. The female-to-male ratio was 1:1.

Efficacy of various doses of silymarin against chemotherapy-induced oral mucositis

Effect of silymarin on body weight

Body weight was measured daily in all experimental groups from day 0 to day 14. No significant changes in body weight were observed in the SM-treated groups, whereas a significant reduction in body weight was detected in the control groups. A highly significant difference was noted between the SM-treated groups and the control groups, as shown in Table 1.

Effect of silymarin on food intake

Food intake was measured daily throughout the experimental period. The daily average food intake in groups II and III was slightly reduced, although the decrease was not statistically significant. In contrast, groups I, IV and V showed significantly lower food intake ($p < 0.05$). Comparison of groups II and III with the control groups demonstrated a highly significant difference (Table 1).

Macroscopic evaluation of chemotherapy-induced oral mucositis

Macroscopic appearance of oral mucositis ulcers

The left buccal mucosa of the rats was subjected to the intraperitoneal administration of 5-FU followed by mechanical injury, resulting in the development of visible ulcerative OM lesions. Random assignments were made to animals with a high macroscopic analysis score on the left side of their cheek pouch. Representative

Table 1. Comparison of body weight and food intake among the experimental groups

Day	Group I		Group II		Group III		Group IV		Group V	
	body weight	food intake	body weight	food intake	body weight	food intake	body weight	food intake	body weight	food intake
0	285 ±3.2	500 ±0.0	288 ±3.1	500 ±0.0	284 ±3.3	500 ±0.0	286 ±3.2	500 ±0.0	287 ±3.1	500 ±0.0
6	278 ±4.9	380 ±84.9*	285 ±2.1*	388 ±79.2*	281 ±2.1*	406 ±66.5**	274 ±8.4	380 ±84.9	267 ±14.1	364 ±96.2
10	269 ±11.3*	274 ±159.8*	282 ±4.2*	377 ±87.0**	280 ±2.8*	384 ±82.1**	230 ±39.6	220 ±198.0	221 ±46.6	154 ±244.7
14	267 ±12.7*	252 ±175.4*	281 ±4.9*	369 ±92.6**	279 ±3.5*	375 ±88.4**	220 ±46.6	133 ±259.5	218 ±48.8	128 ±263.0

Data presented in grams [g] as mean ± standard deviation (*M ±SD*); * statistically significant compared with the control groups ($p < 0.05$, one-way ANOVA followed by Duncan's multiple range test); ** highly statistically significant compared with the control groups ($p < 0.01$, ANOVA).

macroscopic findings are shown in Fig. 2A–E. In group I (SM 2 mg/kg), only mild ulceration was observed, and the lesions healed rapidly. In groups II and III (SM 4 mg/kg and 6 mg/kg, respectively), no mucosal ulceration was detected, indicating a protective effect of SM (Fig. 2A–C).

In contrast, the control groups (group IV, DMSO vehicle control, and group V, 5-FU only) exhibited severe clinical manifestations of OM, including erythema, extensive ulceration and abscess formation. Both groups showed marked mucosal ulceration following 5-FU injection in the absence of SM pre-treatment (Fig. 2D,E).

Oral mucositis ulcer area

The dimensions of oral mucositis ulcers were measured using a digital caliper. Measurements were performed from day 3 to day 14 in the control groups and from day 11 to day 14 in the treatment groups. A highly significant reduction in ulcer area was observed in the SM-treated groups (I–III) compared with the control groups ($p < 0.01$). No significant differences in ulcer area were observed between the 2 control groups during the study period (Table 2).

Histopathological findings

Histopathological examination demonstrated the architecture of the left (injured) and right (non-injured) buccal mucosae in all experimental groups (Fig. 3A–J).

Group I: 2 mg/kg of silymarin

This group received the lowest dose of SM. Histopathological examination of the left buccal mucosa revealed vascular congestion and infiltration of acute inflammatory cells without evidence of ulceration (Fig. 3A). The right buccal mucosa exhibited normal histological architecture (Fig. 3B).

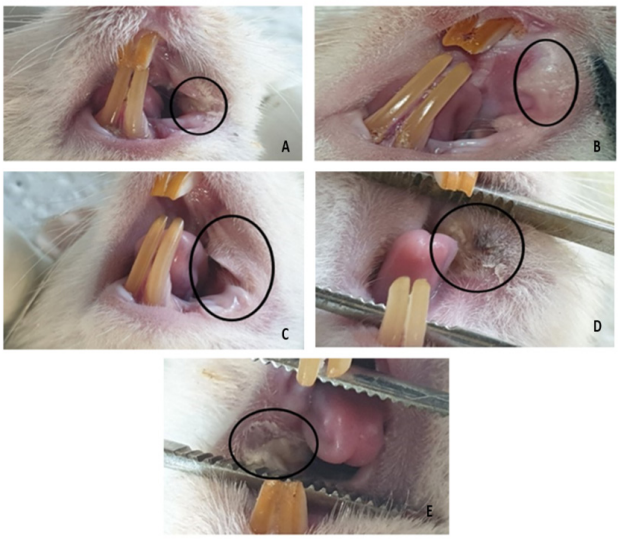


Fig. 2. Representative macroscopic appearance of the buccal mucosa in the experimental groups on day 14

A. Group I; B. Group II; C. Group III; D. Group IV; E. Group V.

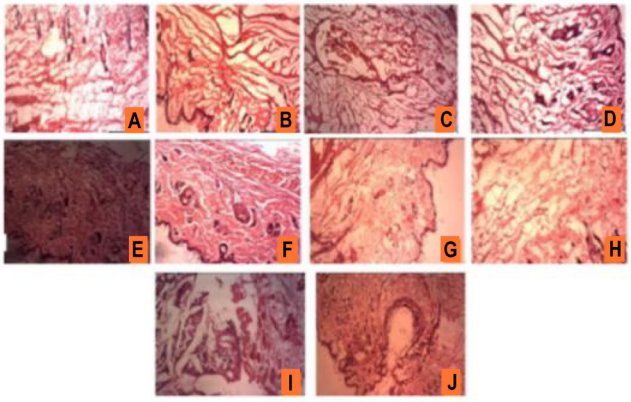


Fig. 3. Representative histopathological images of the buccal mucosa in the experimental groups

A. Group I – left buccal mucosa; B. Group I – right buccal mucosa; C. Group II – left buccal mucosa; D. Group II – right buccal mucosa; E. Group III – left buccal mucosa; F. Group III – right buccal mucosa; G. Group IV – left buccal mucosa; H. Group IV – right buccal mucosa; I. Group V – left buccal mucosa; J. Group V – right buccal mucosa.

Table 2. Comparison of oral mucositis (OM) ulcer area among the experimental groups

Day	Ulcer area [mm^2]				
	group I	group II	group III	group IV	group V
4	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	28.9 ±5.9
6	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	29 ±6.9
8	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	30 ±7.6
10	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	32 ±6.97
11	7.8 ±5.4**	0.0 ±0.0	0.0 ±0.0	29 ±6.2	32 ±7.9
12	9 ±5.7**	4.5 ±3.5**	0.0 ±0.0	30 ±6.4	32 ±7.96
13	9.6 ±6.4**	4 ±3.4**	0.0 ±0.0	31 ±7.5	32 ±7.97
14	10 ±5.8**	2 ±3.6**	0.0 ±0.0	32 ±7.8	32 ±8.3

Data presented as *M ±SD;* ** highly statistically significant compared with the control groups ($p < 0.01$, one-way ANOVA followed by Duncan's multiple range test).

Group II: 4 mg/kg of silymarin

The left buccal mucosa showed vascular congestion with mild inflammatory cell infiltration (Fig. 3C), whereas no histopathological abnormalities were observed in the right buccal mucosa (Fig. 3D).

Group III: 6 mg/kg of silymarin

This group received the highest dose of SM. The left buccal mucosa demonstrated only mild inflammatory cell infiltration without ulcer formation (Fig. 3E). The right buccal mucosa showed normal histological features with no evidence of inflammation or ulceration (Fig. 3F).

Group IV: DMSO vehicle control

The left buccal mucosa showed marked infiltration of acute inflammatory cells, vascular congestion, ulceration, necrosis, and mild fibrosis of the subepithelial layer (Fig. 3G). The right buccal mucosa demonstrated moderate inflammatory cell infiltration accompanied by mild fibrosis (Fig. 3H).

Group V: 5-FU control

The left buccal mucosa exhibited extensive ulceration, necrosis, and dense inflammatory cell infiltration (Fig. 3I). The right buccal mucosa also showed ulceration and necrosis, although inflammatory cell infiltration was less pronounced (Fig. 3J).

Calprotectin and S100A12/EN-RAGE levels

Calprotectin

Groups I, II and III showed no significant changes in plasma CP levels compared with baseline. In contrast, plasma CP levels increased significantly in the control groups (groups IV and V) ($p < 0.05$), as shown in Fig. 4A.

Dose-response analysis demonstrated that treatment with SM reduced plasma CP levels in a dose-dependent manner. The greatest reduction was observed in group III, followed by groups II and I. Analysis of variance demonstrated highly significant differences among the experimental groups ($p < 0.01$).

S100A12/EN-RAGE

Pre-treatment with SM at a dose of 6 mg/kg (group III) resulted in a highly significant reduction in plasma S100A12/EN-RAGE levels compared with baseline ($p = 0.0001$) and with the control groups (95% *CI*, $p = 0.0002$). There was an important growth in group I and a slight non-significant increase in group II compared to the baseline (Fig. 4B).

Analysis of variance demonstrated highly significant differences among the experimental groups ($p < 0.01$). The strongest treatment effect was observed in group III, followed by groups II and I, indicating a dose-dependent effect of SM on plasma S100A12/EN-RAGE levels.

Correlation analysis

Pearson's correlation coefficient (r) was used to evaluate the relationships between plasma biomarker CP and S100A12/EN-RAGE levels and the studied parameters, including body weight, food intake, ulcer area, and the correlation between the 2 biomarkers (Fig. 5).

Correlation of calprotectin with body weight and food intake

There was a non-significant negative correlation between plasma CP levels and body weight ($r = -0.527$, $p = 0.110$) (Fig. 5A). Similarly, plasma CP levels showed a non-significant negative correlation with food intake ($r = -0.927$, $p = 0.060$) (Fig. 5B).

Correlation of S100A12/EN-RAGE with body weight and food intake

Plasma S100A12/EN-RAGE levels demonstrated a strong negative correlation with body weight ($r = -0.756$, $p = 0.070$), although the association was not statistically significant (Fig. 5C).

Likewise, plasma S100A12/EN-RAGE levels showed a very strong negative correlation with food intake

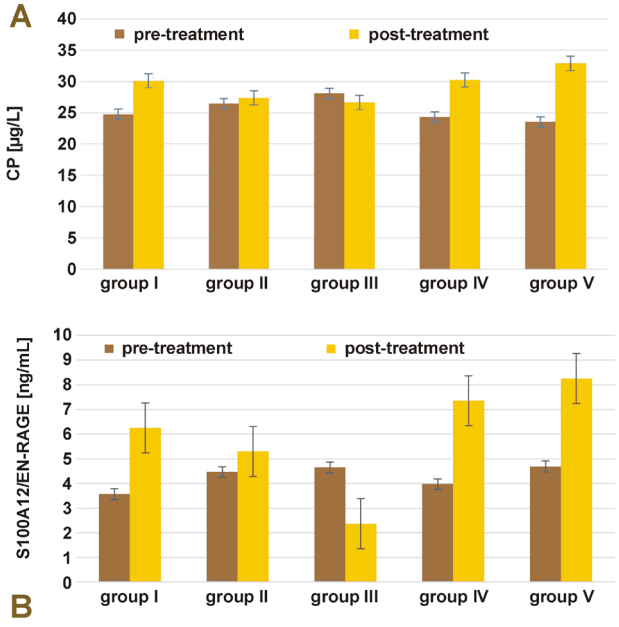


Fig. 4. Plasma levels of inflammatory biomarkers in the experimental groups
A. Calprotectin (CP); B. S100A12/EN-RAGE.

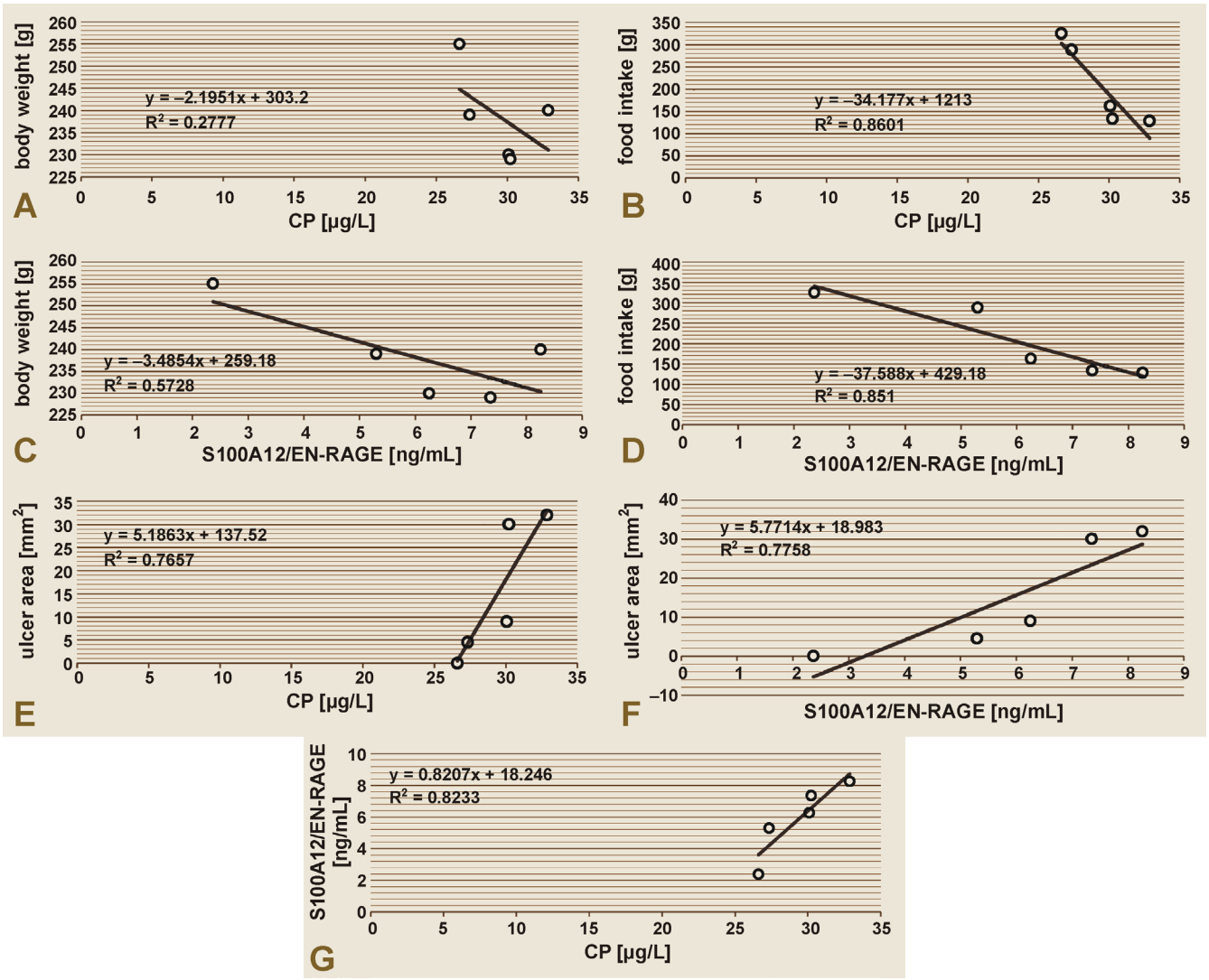


Fig. 5. Correlation analyses between plasma biomarkers and clinical parameters

A. Correlation between body weight and plasma CP levels; B. Correlation between food intake and plasma CP levels; C. Correlation between body weight and plasma S100A12/EN-RAGE levels; D. Correlation between food intake and plasma S100A12/EN-RAGE levels; E. Correlation between mucositis ulcer area and plasma CP levels; F. Correlation between mucositis ulcer area and plasma S100A12/EN-RAGE levels; G. Correlation between plasma S100A12/EN-RAGE and CP levels.

(r = −0.922, p = 0.060), without reaching statistical significance (Fig. 5D).

Correlation of calprotectin and S100A12/EN-RAGE with oral mucositis ulcer area

Plasma CP levels showed a very strong positive correlation with OM ulcer area (r = 0.886, p < 0.0002) (Fig. 5E). Similarly, plasma S100A12/EN-RAGE levels demonstrated a very strong positive correlation with ulcer area (r = 0.887, p < 0.0001) (Fig. 5F).

Correlation between calprotectin and S100A12/EN-RAGE

Pearson's correlation analysis demonstrated a very strong positive correlation between plasma CP and S100A12/EN-RAGE levels (r = 0.907, p < 0.0001) (Fig. 5G).

Discussion

The present study is the first, to our knowledge, to evaluate the efficacy of different doses of SM as a mucoprotective agent against CIOM, while simultaneously assessing the plasma levels of inflammatory biomarkers CP and S100A12/EN-RAGE in an experimental animal model. Oral mucositis was induced using 5-FU. Previous studies have shown that 5-FU induces the production of pro-inflammatory cytokines, leading to basal epithelial cell damage, generation of ROS and inhibition of normal mucosal regeneration.[27] The antioxidant and membrane-stabilizing properties of SM may therefore account for its protective effects observed in the present study.[28]

Chemotherapy-induced oral mucositis is one of the most common and clinically significant toxic adverse effects of anticancer therapy, resulting in extensive tissue

damage.[29] Previous studies have demonstrated that weight loss reaches its maximum between days 7 and 8 following chemotherapy, suggesting a link between the severity of mucositis and low immunological competence with weight loss.[30] In the present study, body weight was measured daily in all groups throughout the experimental period. No significant changes in body weight were observed in the SM-treated groups, whereas significant weight loss was observed in the control groups. These results support the mucoprotective effect of SM.

Food intake was only slightly reduced in the SM-treated groups, whereas a significant reduction was observed in groups I, IV and V. These findings indicate the mucoprotective properties of SM, the efficacy of which was dose-dependent.

Regarding the development of OM, the severity of OM and ulcer size decreased progressively with increasing doses of SM. Rats treated with 2 mg/kg SM developed only mild ulcerative lesions, whereas no ulceration was observed in the groups receiving 4 mg/kg or 6 mg/kg, indicating a dose-dependent mucoprotective effect of SM.

Chemotherapeutic agents such as 5-FU interfere with DNA production primarily in rapidly proliferating cells, including oral epithelial cells. Consequently, mucosal injury is characterized by increased epithelial cell apoptosis and necrosis, accompanied by impaired epithelial regeneration.[31] Consistent with these mechanisms, histopathological examination of SM-treated groups revealed only vascular congestion and mild inflammatory cell infiltration without ulcer formation. In contrast, the control groups exhibited severe inflammatory infiltration, vascular congestion, ulceration, necrosis, and mild subepithelial fibrosis.

Generation of ROS following treatment with chemotherapeutic agents such as 5-FU is considered a key even in the pathogenesis of OM, as demonstrated in numerous previous studies.[32] After the administration of SM, attenuation of mucosal injury was observed. As a potent antioxidant, SM scavenges free radicals and reduces oxidative damage by modulating antioxidant defense systems and inflammatory mediators.[32] Furthermore, SM is well-known for its antioxidant and chemoprotective effects on the liver.[33]

The antioxidant properties of SM promote tissue repair by stimulating RNA and protein synthesis. Silymarin also exhibits immunomodulatory and anti-inflammatory properties through inhibition of T-cell proliferation, suppression of NF-κB activation, and reduction of serum levels of inflammatory cytokines.[34] The reduced inflammatory cell infiltration observed in the SM-treated groups is consistent with these biological activities and further supports the protective role of SM against chemotherapy-induced mucosal injury.

Dimethyl sulfoxide has been widely used as a pharmaceutical solvent, cryoprotectant and penetration enhancer, and has also been investigated for its potential therapeutic applications in oncology and tissue injury. In addition, it can be utilized in the palliative care and pain management sectors.[35] In the current study, DMSO alone did not reduce the severity of OM, indicating that the observed protective effects were attributable to SM rather than the solvent vehicle.

Calprotectin is a neutrophil-derived inflammatory protein whose plasma concentration increases markedly in inflammatory and infectious conditions.[36] The increase in the level of CP depends on the presence of inflammation. When leukocytes become stimulated as a direct result of pathology in different organs, the CP is released, leading to elevated concentrations in plasma and other biological fluids. Calprotectin has become an established biomarker of inflammation.[37] In the present study, plasma CP levels significantly increased in the control groups due to the inflammation and mucositis but remained unchanged in the SM-treated groups. Furthermore, CP concentrations were significantly lower in the SM-treated groups than in the controls. These findings suggest that SM has protective effects on the mucous membrane, preventing inflammation and CIOM.

The association of S100A12 with the multi-ligand RAGE receptor and the sRAGE plays a vital role in pro-inflammatory pathways. In different inflammatory conditions, S100A12 is essential in local inflammatory responses, and serum S100A12 levels are elevated in chronic inflammatory diseases.[38] Our findings are consistent with these observations. Plasma S100A12/EN-RAGE levels increase significantly in the control groups, whereas pre-treatment with 6 mg/kg SM resulted in a marked reduction compared with both baseline and the control groups. These results further support a dose-dependent anti-inflammatory effect of SM in experimental CIOM.

Correlation analysis demonstrated negative, although non-significant, associations between plasma CP and S100A12/EN-RAGE concentrations and both body weight and food intake. In contrast, both CP and S100A12/EN-RAGE levels showed very strong positive correlations with OM ulcer area. These findings suggest that both biomarkers reflect the severity of mucosal injury. An additional important finding was a highly significant correlation between plasma CP and S100A12/EN-RAGE levels.

Previous studies have shown that SM reduces inflammation and suppresses the production of pro-inflammatory cytokines.[39] Bioactive constituents of *S. marianum* play a significant role in a wide range of pathological conditions, such as cancer.[40] The findings of the present study are consistent with these reports and suggest that the mucoprotective effects of SM may be associated with attenuation of inflammation and oxidative stress, accompanied by reduced plasma CP and S100A12/EN-RAGE levels. These observations provide evidence for a potential additional mechanism underlying the protective effects of SM against CIOM.

Conclusions

Silymarin exerts dose-dependent mucoprotective effects against CIOM, which are associated with reduced CP and S100A12/EN-RAGE levels. Pre-treatment with SM attenuated inflammation and reduced the development of oral mucositis, as confirmed by both macroscopic and histopathological analyses. Furthermore, the very strong positive correlations between plasma CP and S100A12/EN-RAGE levels and OM ulcer area suggest that these biomarkers may have potential clinical value for assessing the severity of CIOM.

Ethics approval and consent to participate

The study protocol was approved by the Institutional Committee for Ethics in Animal Use at the Faculty of Pharmacy, Isra University, Amman, Jordan (registration No. 3-24/2019), and was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

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

Oral lichen planus affecting the gingiva is associated with an increased incidence of aggressive periodontal pathogens: A comparative study

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Abstract

Background. Chronic inflammation in the oral cavity has a negative effect on the course of chronic mucosal lesions.

Objectives. The present study aimed to compare the volume and proportional presence of aggressive periodontal pathogens in patients with oral lichen planus (OLP) accompanied by desquamative gingivitis (DG) vs. those with OLP without gingival involvement. All patients in the cohort were also diagnosed with moderate chronic periodontitis. The observed differences may help explain the higher risk associated with gingival involvement in OLP.

Material and methods. The presence of periodontal pathogens was evaluated in 50 biopsy-confirmed OLP with DG cases and 50 OLP cases without gingival manifestations. All participants presented with chronic periodontitis of comparable severity (moderate: periodontal pocket depth (PPD) up to 6 mm). Aggressive periodontal pathogens were identified and quantified using DNA testing (VariOr®-Dento). The periodontal status was assessed using orthopantomography (OPG) or cone-beam computed tomography (CBCT), in combination with the clinical examination of periodontal pockets.

Results. In comparison with the non-gingival OLP cases, the OLP cases with gingival involvement exhibited a significantly higher proportional presence of aggressive periodontal pathogens ($p = 0.0009$) and a higher bacterial load ($n/\mu L$; $p = 0.001$). Desquamative gingivitis associated with OLP was also linked to an increased risk of subsequent periodontal resorption. Given the unstable chronic inflammatory environment, careful monitoring of periodontal tissues in OLP cases appears to be essential, as persistent inflammation may contribute to an elevated risk of malignant transformation.

Conclusions. Our findings demonstrate a significant difference in the presence of aggressive periodontal pathogens between OLP cases with and without gingival involvement. The median pathogen volume in the OLP with DG cases was over 7.4 times higher. Chronic inflammation and bacterial by-products may act as cofactors in the development of dysplasia and the malignant transformation of oral lichen with dysplasia (OLD), which may present clinically as DG.

Keywords: oral lichen planus, periodontal pathogens, oral potentially malignant disorder, desquamative gingivitis, DNA testing

Highlights

- Gingival oral lichen planus (OLP) is associated with a more severe burden of aggressive periodontal pathogens.
- Chronic inflammation may act as a cofactor in promoting oral epithelial dysplasia.
- The study demonstrates OLP with desquamative gingivitis (DG) cases to be at risk of malignant transformation.

Introduction

Oral lichen planus (OLP) is a common chronic inflammatory disease of the oral mucosa, with an incidence of approx. 2%.[1–4] It is characterized by white reticular (lace-like) patches and atrophic or erosive (ulcerative) lesions.[1–3] Oral lichen planus, which typically affects females over 40 years of age, with a female-to-male ratio of 1.4:1, was first described by Wilson in 1869.[2] Its clinical course is often unstable, featuring multiple remissions and relapses. Gingival involvement may present as desquamative gingivitis (DG), while lesions on the dorsum of the tongue often appear as plaque-like patches. Desquamative gingivitis is characteristic gingival involvement in OLP, both localized and generalized. Gingival lesions exhibit a spectrum of clinical manifestations, ranging from hyperkeratosis and atrophy to the erosive destruction of the gingival epithelium.[5–8]

Various clinical manifestations of OLP are shown in Fig. 1. White lesions are usually asymptomatic, whereas ulcerative lesions may cause a burning sensation and pain.[3,4] Mucosal alterations are the result of the disrupted epithelial regeneration following basal cell destruction.[5] This process is associated with a lichenoid subepithelial infiltrate composed of cytotoxic T-lymphocytes (cluster of differentiation: CD8+).[5,9] The clinical severity of OLP is often assessed using the reticular-erythematous-ulcerative (REU) index,[10] and subjective symptoms are measured using the visual analog scale (VAS).[10,11] In contrast to bilateral OLP, oral lichenoid lesions (OLL) are typically unilateral or asymmetrical.[1,6,12] Oral lichenoid lesions include oral contact lesions, lichenoid drug eruptions, and the oral manifestations of graft-versus-host disease (GvHD).[6,13,14]

Histopathological criteria for OLP include a subepithelial lymphocytic infiltrate, parakeratosis and acanthosis.[1,4,6,13,15,16] Biopsy is essential to distinguish non-dysplastic OLP from OLP with dysplasia (OLD).[1,9,17] Direct and indirect immunofluorescence are valuable tools for differentiating OLP from autoimmune bullous diseases.[8,18–21] Immunohistochemistry further enhances diagnostic accuracy, often through the analysis of proteins such as p53 and Ki-67.[7,22,23] Mutated p53 protein is typically found in the nuclei of dysplastic or neoplastic epithelial cells.[23–25] In differentiated oral intraepithelial neoplasia (dOIN), p53 is detected along a continuous row of nuclei in the epithelial basal layer. Ki-67 serves as a marker for cellular proliferation.[26]

Oral lichen planus has been classified as a potentially precancerous condition since 1978. More than a century has passed since the first documented case of malignant transformation to oral squamous cell carcinoma (OSCC) in OLP.[7] Malignant transformation is driven by increased epithelial proliferation and inflammation-mediated carcinogenic processes.[27,28] A 2019 systematic review estimated the risk of transformation at 0–3.5%.[29] However, a more recent one reported a lower risk of 0.44% after excluding cases with less than 6 months between OLP biopsy and carcinoma diagnosis.[27] Risk factors for OLP progression include the presence of oral epithelial dysplasia (OED) and *Candida albicans* infection.[7,30] Clinically, the erosive form carries the highest risk.[13]

Since early lesions may resemble flat leukoplakia, and lichenoid infiltrates are present in biopsy, the more serious diagnosis of proliferative verrucous leukoplakia (PVL) may be mistakenly identified as OLP.[7] The rate of malignant transformation in PVL ranges from 50% to 100%.[29] If lesions meet the Cerero-Lapiedra criteria for PVL, a more intensive follow-up is warranted to minimize the risk of malignant transformation (Fig. 1).[31]

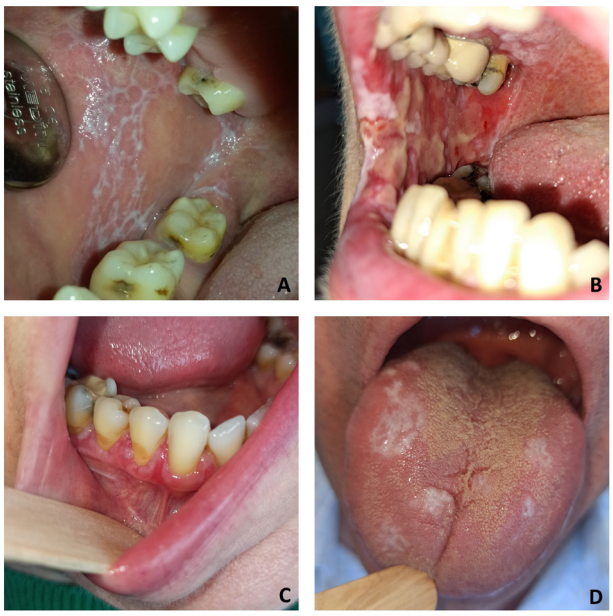


Fig. 1. Clinical manifestations of oral lichen planus (OLP)

A – reticular OLP on the buccal mucosa is the most frequent clinical form (female, 61 years); B – atrophic-erosive form on the buccal mucosa brings severe subjective complaints (female, 65 years); C – desquamative gingivitis (DG) was present in over 40% of our patients (female, 72 years); D – the plaque form affecting the dorsum of the tongue is a less frequent white OLP manifestation (male, 62 years).

The etiology of OLP is usually unknown.[1] A time delay of 6–12 months between the triggering factor and the clinical manifestation is often observed.[32,33] Common comorbidities of OLP include immune-mediated liver and thyroid gland diseases and cutaneous lichen planus (CLP).[34–38]

Topical treatment for OLP includes corticosteroids in gel form, mucosal adhesive patches and laser therapy.[39–42] The surgical excision of the most clinically severe lesions is often a necessary part of the therapeutic protocol. In suspected cases of drug-induced lichenoid reactions, clinicians are advised to modify the patient's medication regimen.

The relationship between periodontal tissues and OLP manifestations remains unclear. The inflammatory process in periodontitis is strongly associated with aggressive periodontal pathogens.[43] In the oral cavity, anatomical proximity allows even localized infections to exert widespread pathological effects. The most aggressive bacteria involved in periodontitis are members of the so-called "red complex". These pathogens – *Porphyromonas gingivalis* (*Pg*), *Tannerella forsythia* (*Tf*), *Treponema denticola* (*Td*), and *Filifactor alocis* (*Fa*) – are implicated in alveolar bone resorption through inflammatory mechanisms.[43] The virulence of these pathogens is directly correlated with their concentration. The members of the red complex are often accompanied by other aggressive species belonging to the orange complex, including *Parvimonas micra* (*Pm*), *Prevotella intermedia* (*Pi*) and *Fusobacterium nucleatum* (*Fn*).[43,44] These bacteria produce proteases and other cytotoxic by-products.

Numerous studies have demonstrated that such bacteria not only induce inflammation, but also contribute to the development of epithelial dysplasia, thereby increasing the risk of malignant transformation. These microorganisms can inhibit apoptosis in atypical cells, further contributing to carcinogenesis.[45,46] Periodontal pathogens, such as *Fn*, have been implicated in the development of the cancers of the pancreas and the intestine by promoting inflammation, cellular proliferation and immune evasion.[47] Similarly, *Tf* and *Pg* have been linked to esophageal cancer.[45,48] Biopsies of OSCC have frequently been found to contain high levels of *Pg* contamination.[45] Bacterial by-products include matrix metalloproteinases (MMPs) – zinc-dependent peptidases that degrade the extracellular matrix and contribute to the disruption of the basement membrane.[49,50] Antibiotics such as amoxicillin, in combination with metronidazole or tetracycline, are often prescribed as adjunctive therapy for periodontitis.[44,51] Differences in antibacterial and anti-MMP efficacy depend on dosage and treatment duration.[52–54]

The aim of this study was to compare the periodontal pathogen profile in OLP patients with DG and in OLP cases without DG, matched by sex, age and periodontitis severity (the same periodontal pocket depth (PPD), the same periodontitis stage and grade). This comparison may help elucidate the differential interaction of aggressive microorganisms and their by-products with the host immune system. Such mechanisms could explain the higher rate of malignant transformation observed in cases of OLD in alveolar localization. Chronic inflammation, the presence of cytotoxic bacterial by-products and the formation of carcinogenic compounds may drive excessive epithelial proliferation, ultimately contributing to the development of OSCC within OLP lesions.

Material and methods

Between 2004 and 2025, a total of 454 biopsy-verified cases of OLP or OLD were diagnosed and managed at the Department of Stomatology-Oral Medicine, University Hospital Pilsen, Czechia. Each case underwent a subsequent immunohistochemical examination. Clinical evaluations included radiographic examinations, the assessment of tobacco use, REU scoring, and VAS profiling. Regular swab cultures for *Candida albicans* from the oral mucosa were also performed.

Oral lichen planus/OLD was diagnosed through routine biopsy, supplemented by direct immunofluorescence, which identified fibrinogen deposits along the basement membrane. Immunohistochemistry was used to evaluate p53 and Ki67 expression.

The inclusion criteria were as follows: OLP cases verified by a clinical examination with an REU evaluation and differentiation by the affected mucosal sites, with mandatory confirmation via biopsy. All biopsies were followed by direct immunofluorescence. The patients who had not taken antibiotics or undergone oral surgery in the previous 4 months were included. Only OLP cases with the co-existing chronic periodontitis were included (defined by the 1999 classification as periodontal pockets with an average depth of 6 mm and radiographic evidence of bone resorption, or Stage III, Grade B periodontitis by the 2017 classification). The cases of OLP with DG were sex- and age-matched to the patients with OLP without gingival involvement.

The exclusion criteria comprised OLP cases not confirmed by biopsy; the cases showing features of aggressive periodontitis or the acute exacerbations of chronic periodontitis (e.g., periodontal abscess or excessive bleeding on probing, Stage I, II, IV and Grade A, C); the use of antibiotics or oral surgery within the last 4 months; and OLP cases not matched by sex and age to the study cohort.

The study compared 50 patients with OLP with DG to 50 patients with OLP without gingival involvement. The sex ratio matched the overall clinic population (2.2:1), resulting in 34 females and 16 males in each group. The average age of the participants was 62.55 years (range: 31–85 years). Patients with OLP with DG were classified as group 1 (G1), and those with non-gingival OLP formed group 2 (G2). All participants provided written informed consent. The characteristics of the comparative study cohort are presented in Table 1.

Table 1. Comparative study cohort – sex ratio and age

Group	Sex F/M	Mean age [years]	Age range [years]
G1: OLP with DG	34/16	62.5	31–83
G2: OLP without DG	34/16	62.6	33–85

F – female; M – male; OLP – oral lichen planus; DG – desquamative gingivitis.

The diagnosis of chronic periodontitis was established clinically using a WHO periodontal probe. Bone resorption was confirmed radiographically via orthopantomography (OPG) or cone-beam computed tomography (CBCT). Periodontal pocket depth ranged from 5.5 to 6 mm. Tooth mobility was assessed through manual palpation, which was a mandatory component of the clinical evaluation.

The detection of aggressive periodontal pathogens belonging to the red and orange complexes was performed using a DNA-based test on the subgingival plaque collected with sterile paper points. The VariOr®-Dento Plus system (GenTrend Co., Rudolfov, Czech Republic) was used to identify 12 periodontal pathogens. The results were reported both as proportions (scale 0–4) and as bacterial load (in microliters). A proportional value of 4 corresponds to approx. 10^6–10^7 bacteria, while a value of 1 equates to 10^2–10^3. Higher values indicate worse prognosis in terms of periodontal tissue resorption. DNA detection was conducted using the real-time quantitative polymerase chain reaction (qPCR) with TaqMan™ probes, with a detection threshold of 10^1.

Statistical analysis

Statistical analysis was performed using the SigmaXL software (SigmaXL Inc., Kitchener, Canada). Non-parametric tests, including the Kruskal–Wallis test, were applied for group comparisons.

Results

The cohort included in the comparative study comprised 454 patients diagnosed with OLP/OLD. Of these, 60% were classified as OLP (n = 274/454) and 40% as OLD (n = 180/454). The average follow-up period was 63.1 months (5.26 years). The female-to-male ratio was 2.2:1 (n = 314 and n = 140, respectively). The mean age at the time of the study was 63.1 years. Gingival involvement (DG) was observed in 43% of cases (n = 195/454). The mean REU index across the cohort was 10.1, with higher values in the DG cases (13.9) as compared to those without gingival manifestations (7.4). The erosive-ulcerative form of OLP was more frequently associated with gingival involvement (47% vs. 33% in non-gingival OLP). The mean VAS score for pain was slightly higher in the DG cases (1.44) as compared to the non-gingival cases (1.09).

Only 19.6% of the patients were smokers (n = 89/454), with no difference in prevalence between the DG cases and OLP without gingival manifestations. Concurrent thyroid disease was found in 19.8% of the OLP/OLD cases (n = 90/454). The mean colony-forming unit (CFU) count of *Candida albicans* was notably higher in patients with OLP with DG (17.1) than in the non-gingival OLP cases (10.3).

The immunohistochemical analysis of the p53 protein was performed in 48.2% of cases (n = 219/454). Nuclear positivity for p53 in long rows of basal epithelial cells was present in 50% of the DG cases (n = 44/88) and 59% of the non-gingival cases (n = 77/131). Due to limited sampling (<5% of cases), Ki67 expression did not provide a statistically meaningful diagnostic insight.

Differentiated oral intraepithelial neoplasia was diagnosed with similar frequency in both groups (80% in DG vs. 81% in non-gingival OLD). The malignant transformation rates were also comparable between the OLP cases with DG (2.4%, n = 3/125) and those without (2.0%, n = 3/149). However, among the OLD cases, the rate of malignant transformation was significantly higher in the DG cases (11.4%, n = 8/70) than in non-gingival OLD (7.3%, n = 8/110).

A summary of the clinical-pathological characteristics of both OLP variants is presented in Table 2.

Topical corticosteroids – dexamethasone acetate and triamcinolone acetonide – were used to treat mild to moderate cases of OLP. In severe cases, clobetasol propionate was administered. The formulation was selected based on the location of the lesion; solutions and gels were optimal for the buccal mucosa and the tongue, while mucosal adhesive pastes were used to treat DG. Among the patients without DG, 56.8% (n = 147/259) required corticosteroid therapy as compared to 74.9% (n = 146/195) of those with OLP and DG. Over 90% of topical applications involved dexamethasone, with the remainder using clobetasol.

Surgical treatment included diagnostic excision at the initial OLP follow-up visit, with the re-excision performed in cases of clinical lesion progression. In instances of gingival desquamation, the adjacent teeth were extracted when malignant transformation was suspected. This approach ensured the removal of all at-risk tissue.

Table 2. Clinical-pathological differentiation between oral lichen planus (OLP) variants

Parameter	OLP	OLP with DG
Number of cases (n)	259	195
REU index	7.4	**13.9**
Erosive-ulcerative OLP [%]	33	**47**
VAS pain score	1.09	**1.44**
p53 nuclear positivity [%]	**59**	50
dOIN [%]	**81**	80
Candida albicans [CFU]	10.3	**17.1**
Malignant transformation in OLP [%]	2.0	**2.4**
Malignant transformation in OLD [%]	7.3	**11.4**

REU index – reticular-erythematous-ulcerative index; VAS – visual analog scale; dOIN – differentiated oral intraepithelial neoplasia; CFU – colony-forming units; OLD – oral lichen planus with dysplasia. Significant differences are shown in bold.

In the patients with OLP and DG, OPG or CBCT scans were performed to evaluate alveolar bone resorption and assess the potential for gingival lesion progression, even before surface abnormalities appeared.

To compare the bacterial profiles, the volume of aggressive periodontal pathogens was assessed in 50 patients with OLP and DG and in 50 patients with OLP without gingival involvement. All patients had a comparable periodontal status, with moderate chronic periodontitis confirmed through the clinical examination and CBCT analysis. The mean PPD in the compared cases was 6 mm, and periodontitis was classified as Stage III and Grade B.

Both the proportional presence and absolute bacterial volumes (in milliliters) of aggressive periodontal pathogens were analyzed. Particular emphasis was placed on pathogens from the red and orange complexes. In both categories, OLP with DG showed a predominance. Specifically, the red complex pathogens – *Pg*, *Tf*, *Td*, and *Fa* – were significantly more prevalent in the DG group. Similarly, the orange complex bacteria such as *Pm* and *Fn* were also more common in OLP with DG. The only pathogen with a slightly lower proportional presence in DG was *Pi*. These differences were statistically significant (p = 0.0009; Kruskal–Wallis test), with confidence intervals (*CI*): 2–3 for OLP with DG, and 1–2 for OLP without DG.

Table 3 summarizes the proportional comparison of the red and orange complex pathogens.

Similar trends were observed for the absolute bacterial volumes. The mean and median bacterial counts (in milliliters) were significantly higher in the OLP with DG group (p = 0.001; Kruskal–Wallis test). The *CI*s were as follows: 55.4k–111.6k for OLP with DG; and 4.3k–21.4k for OLP without DG. On average, the quantity of aggressive periodontal pathogens in OLP with DG was 7.4 times higher than in those without gingival involvement.

The results of both comparisons are presented in Fig. 2 and 3.

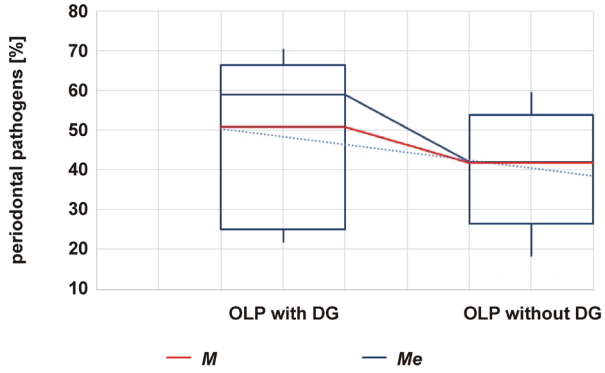


Fig. 2. Correlation between oral lichen planus (OLP) lesion distribution and periodontal pathogens

M – mean; *Me* – median. Periodontal pathogens belonging to the red and orange complexes in significantly different proportions in cases of OLP with gingival manifestations as compared to OLP without gingival involvement.

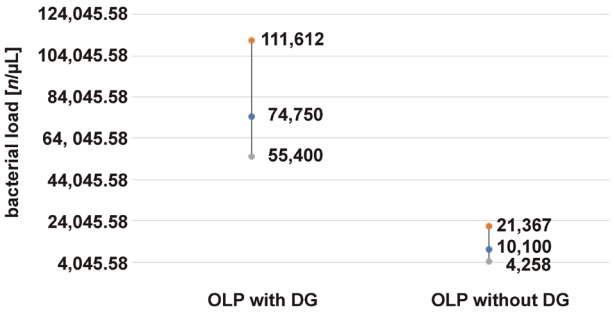


Fig. 3. Association of the periodontal pathogen load with oral lichen planus (OLP) lesion distribution

Periodontal pathogens belonging to the red and orange complexes, detected in different variants of OLP. Cases with DG are associated with a 7.4 times higher load of aggressive bacteria.

Discussion

Oral lichen planus is a common chronic disease of the oral mucosa, with an incidence exceeding 2%.[1–3] It typically affects patients over 40 years of age and shows a slight female predominance, with a female-to-male ratio of approx. 1.4:1.[4] In our study, this ratio was even more pronounced at 2.2:1 in favor of women.

Oral lichen planus frequently coexists with thyroid gland disorders. Studies from Northern Europe and Spain report thyroid involvement in over 8% of OLP cases.[36,37] In our cohort, 19.8% of patients with OLP or OLD had concomitant pathologies in the thyroid. This higher prevalence may be attributed to our standard practice of referring all OLP patients to a general practitioner for thyroid function screening.

Oral lichen planus was included among the oral potentially malignant disorders (OPMDs) in the 3rd and 4th editions of the World Health Organization (WHO) classification.[7] However, the current 5th edition limits this classification to cases of OLD, reflecting the updated understanding of malignant potential.[7]

Table 3. Proportional comparison [%] of the red and orange complex periodontal pathogens

Disease	*Pg*	*Tf*	*Td*	*Fa*	*Pm*	*Pi*	*Fn*
OLP with DG	**59.0**	**70.5**	**50.5**	**22.0**	**63.5**	25.0	**66.5**
OLP	42.0	54.0	38.5	18.5	53.5	**26.5**	59.5

Pg – Porphyromonas gingivalis; *Tf – Tannerella forsythia*; *Td – Treponema denticola*; *Fa – Filifactor alocis*; *Pm – Parvimonas micra*; *Pi – Prevotella intermedia*; *Fn – Fusobacterium nucleatum*. Higher values are bolded.

A high incidence of dOIN has been observed in OLP patients. Differentiated oral intraepithelial neoplasia is considered a high-risk lesion in some specialized centers.[55] In our study, dOIN was present in 80% of the OLD cases with DG (n = 56/70) and in 81% of the non-gingival OLD cases (n = 89/110).

Data on the expression of p53 and Ki-67 in OLP is conflicting. Some studies link these markers to malignant transformation, while others do not support this association.[22] In our study, p53 positivity was more frequent in the OLP cases without DG (59%) than in those with gingival involvement (50%). Other studies have reported p53 and Ki-67 positivity in over 80% of OLP biopsies.[9]

Historically, the reported rate of malignant transformation in OLP was high, largely due to a failure to distinguish between non-dysplastic OLP and OLD. In 2019, González-Moles et al. estimated the malignant transformation rate for OLP to be between 0% and 3.5%.[29] This was further reduced to 0.44% by Idrees et al. in 2021 after excluding cases suspected of concurrent OLP and OSCC.[27] Using the same exclusion criteria, we found the malignant transformation rate for OLP with DG to be 2.4% (n = 3/125), and for non-gingival OLP, 2.0% (n = 3/149). Both figures fall at the higher end of recent estimates.[27,29] However, the malignant transformation rate was significantly higher in the OLD patients. Transformation to OSCC occurred in 7.3% of the cases without gingival involvement (n = 8/110) and in 11.4% of those with DG (n = 8/70). This increased rate in the DG cases may be attributed to long-standing, intense chronic inflammation of the surrounding tissues. Such inflammatory processes, driven by microbial activity and their by-products, interact with the immune system, influencing tissue repair pathways and dysplasia progression.

Given the risk of misdiagnosing the early lesions of PVL as OLP, all patients with biopsy-confirmed OLP should remain under continuous clinical follow-up.[7,31] Inexperienced clinicians may overlook the subtle changes associated with malignant transformation.[31]

Radiographic imaging should be a routine part of comprehensive care for patients with OLP with DG and those suspected of PVL. Malignant transformation in PVL is notably high, ranging from 50% to 100%.[29] Our findings – an 11.4% malignant transformation rate in OLD with DG – indicate that this condition also carries significant malignant potential.

The OLP therapy typically includes topical corticosteroids, such as dexamethasone acetate and clobetasol propionate.[39,40] In our study, corticosteroids were required in 56.8% of the OLP cases without gingival involvement. The cases presenting with DG were more challenging to manage, requiring corticosteroid treatment in over 74.9% of instances. Laser therapy is also frequently employed in symptomatic OLP cases,[56–58] particularly for ulcerative lesions that do not respond to corticosteroids. However, therapeutic outcomes in OLP are generally inconsistent across studies,[42] and no curative treatment has yet been identified.[4,10]

Functional immune surveillance is essential for good oral mucosal and periodontal health. Defense against pathological processes requires a rational lifestyle, ensuring the intake of nutrients and elements necessary for the functioning of the organism.[59] Periodontal health can be compromised by comorbidities or genetic predisposition to gum disease.[60,61] The periodontal disease classification system has evolved from the specification of the suspected induction to the determination of the risk of sequelae.[62] The association between aggressive periodontal pathogens and diseases or malignancies at both local and distant sites is well-documented.[45–48,63,64] The interactions between these bacteria and the human immune system are complex. Their presence and metabolic by-products perpetuate chronic inflammation, leading to tissue destruction, dysplasia development and dysregulated immune responses. In OLP, this process is further complicated by the cytotoxic activity of the subepithelial lichenoid infiltrate.[5]

The atrophy and erosions associated with DG cause pain and burning sensations, impairing the patient's ability to maintain proper oral hygiene.[10,11] This creates a bidirectional feedback loop – immune-mediated mucosal damage impedes hygiene, promoting plaque accumulation, which in turn fosters aggressive bacterial colonization and immune system activation.

Our results showing a significant prevalence of aggressive perio-pathogens in the OLP cases with DG confirm the bilateral nature of the pathological process. Chronic inflammation associated with the effects of bacterial activity increases the intensity of OLP manifestations, expressed by the REU index and a higher incidence of the erosive form of OLP. The difficult-to-manage course of these cases of lichen requires more frequent use of corticosteroids, resulting in higher contamination of the oral cavity with *Candida* species. Pain and reduced oral hygiene options in severe OLP in turn retrospectively impair periodontal tissue management.

In cases of OLP/OLD with DG, both comprehensive diagnostics and targeted treatment are essential. The risk of dysplasia development in clinically diagnosed OLP lesions remains.[65,66] A well-established link exists between periodontal pathogen activity and oral carcinogenesis.[67] Some researchers have proposed that the ultimate therapeutic goal in OLP might be to "erase the memory" of the affected oral tissue.[68]

To optimize patient care, it is essential to integrate histopathological biopsy findings, radiographic imaging, the clinical assessment of periodontal tissues, and microbial profiling.[69] These diagnostic tools should guide a multifaceted treatment approach aimed at reducing lichenoid infiltrate activity, promoting periodontal healing, and neutralizing the effects of aggressive periodontal pathogens and their virulence factors.

A major challenge in conducting the present study was minimizing the potential bias. To address this, only biopsy-confirmed cases of OLP were included, and patients were

matched by sex, age and the periodontal clinical status. Further large-scale studies exploring the interactions between periodontal bacteria, their by-products and immune responses are needed to improve treatment strategies for chronic oral mucosal lesions.

Conclusions

Our study demonstrates a significant difference in the presence of aggressive periodontal pathogens – specifically those belonging to the red and orange complexes – between cases of OLP with gingival involvement and those with lesions in other oral locations. The median bacterial volume in OLP cases with DG was more than 7.4 times higher. Chronic inflammation, along with the by-products of these aggressive bacteria, may act as cofactors in promoting dysplasia and increasing the risk of malignant transformation in OLD involving DG.

These findings support the need for a comprehensive therapeutic approach that combines agents targeting the cytotoxic activity of the subepithelial lichenoid infiltrate with drugs that have antimicrobial effects. Additionally, cases of OLP and OLD with gingival manifestations should undergo an X-ray examination to reduce the risk of the delayed detection of malignant transformation.

Ethics approval and consent to participate

The present scientific project was approved by the Institutional Review Board (IRB) and Ethics Committee (EC) of the University Hospital Pilsen, Czechia (approval No. 231/24). Written informed consent was obtained from all participants before their inclusion in the study. This study 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

Association between malocclusion severity and adolescents' psychosocial status

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Abstract

Background. The impact of dentoskeletal malocclusion on the psychosocial status and oral health-related quality of life (OHRQoL), especially in adolescents, has been a subject of considerable debate in the literature.

Objectives. The aim of the study was to explore the relationship between the severity of dental and skeletal malocclusions and adolescents' self-esteem, their perception of dentofacial aesthetics, and the psychosocial impact of dental aesthetics.

Material and methods. Adolescent patients (aged 10–18 years) seeking orthodontic treatment in a hospital clinic were recruited for the study. The Psychosocial Impact of Dental Aesthetics Questionnaire (PIDAQ), the Orofacial Esthetic Scale (OES), the Rosenberg Self-Esteem Scale (RSES), and the Aesthetic Component of the index of orthodontic treatment need (IOTN-AC) were administered during the first consultation. Overbite (OVB), overjet (OVJ) and the Dental Health Component of the IOTN (IOTN-DHC) were measured by a trained operator on digital models. Lateral cephalometric radiographs were used to evaluate skeletal malocclusions in the sagittal and vertical planes. Comparisons between the groups were performed using analysis of variance (ANOVA), the Kruskal–Wallis test or the χ^2 test. Correlations between variables were assessed using Pearson's or Spearman's correlation tests.

Results. The majority of adolescents classified according to the IOTN-DSC as having a definite need for orthodontic treatment by clinicians perceived their own need for treatment as low or non-existent. No statistically significant differences were found in the PIDAQ, OES and RSES scores among different IOTN-DHC subgroups. Overjet was associated with self-esteem, while OVB was correlated with dental self-confidence and overall assessment of orofacial aesthetics. Skeletal variables were not associated with the studied domains. The PIDAQ was significantly positively correlated with the IOTN-AC and negatively correlated with the RSES.

Conclusions. The perception of malocclusion severity differs between patients and clinicians, highlighting the need to consider patients' values when defining orthodontic goals. Overjet and OVB are clinical variables that can influence the psychosocial domain in adolescents.

Keywords: self-esteem, malocclusion, oral health-related quality of life, psychosocial impact, orofacial aesthetics

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Highlights

- Adolescents' self-perceived need for orthodontic treatment (IOTN-AC) is substantially lower than the normative need assessed by clinicians (IOTN-DHC); most patients classified as having a definite treatment need rate their own need for orthodontic treatment as low or non-existent.
- Normative malocclusion severity and skeletal cephalometric parameters are not associated with adolescents' self-esteem, psychosocial impact of dental aesthetics, or self-perceived orofacial aesthetics.
- Overjet and overbite are the only clinical variables associated with psychosocial outcomes; overjet relates to self-esteem, whereas overbite relates to dental self-confidence and orofacial aesthetics.
- Patients' subjective perceptions and expectations should be integrated into orthodontic treatment planning through shared decision-making and patient-reported outcome measures.

Introduction

In modern society, an attractive physical appearance has a positive impact on social relationships. Individuals who are considered more physically attractive tend to have more opportunities for social interaction and acceptance, which has a positive effect on their social life, both in terms of friendships, romantic encounters and professional opportunities.[1,2] Physical appearance and an attractive smile play an important role in the lives of adolescentsand young adults.[3,4] Adolescence is characterized by alterations in personality, psychological adaptability, as well as physical changes throughout pubertal development.[5] As a result, adolescents may base their self-perception on physical characteristics such as weight and facial features.

Given the pivotal role of the dentition in smile aesthetics, it is reasonable to expect that severe malocclusion can influence an adolescent's psychosocial status. Krekmanova et al. reported that adolescents seek orthodontic treatment mostly to enhance their aesthetic appearance and social acceptance.[6] The perceived emotional and social effects of dental appearance in adolescents are closely linked to the severity of malocclusion.[7] However, a notable difference exists between the aesthetic perceptions of clinical experts and patients, which can become a significant issue.[8] Therefore, when clinicians decide on the necessity of orthodontic treatment, they should take into account patients' perceptions of malocclusion severity and its impact on emotional and social well-being.[9] The evaluation of psychosocial well-being frequently employs quality of life (QoL) assessment tools. According to the World Health Organization (WHO), QoL is defined as "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns".[10] In the realm of oral health, QoL is commonly referred to as oral health-related quality of life (OHRQoL), representing the effect of oral health on a person's overall QoL.[11]

Various studies have reported that severe malocclusions have a negative impact on OHRQoL.[8,12] In particular, Dimberg et al. suggested that malocclusions in the aesthetic zone can have a negative impact on OHRQoL in children and adolescents, particularly on their emotional and social well-being.[13] Previous literature has shown that the greater the deviation from average overjet (OVJ)[14,15] and overbite (OVB)[15] values, the lower the OHRQoL perceived by adolescent patients. Moreover, perceptions of individuals with certain dental anomalies, such as a midline diastema and a gummy smile, change significantly before and after orthodontic treatment.[16] In addition to dental malocclusions, skeletal malocclusions also significantly influence OHRQoL. In a pilot study conducted by Pereira et al., significant differences were found in the psychological discomfort domain of OHRQoL depending on the severity of skeletal malocclusion.[17] Individuals with hyperdivergent facial types tend to report lower OHRQoL compared to those with normodivergent facial types, particularly in aspects related to social interactions.[18] However, according to a recent systematic review, there is limited evidence that malocclusions in adolescents negatively affect their OHRQoL.[19] Similarly, Kolawole and Ayodele-Oja found that malocclusion severity did not affect OHRQoL.[20]

Additionally, it has been reported that the severity of malocclusion moderately influences dental aesthetics-related OHRQoL, which indicates that malocclusion may not be the only determining factor in an individual's perception of dental aesthetics.[12] Spilker emphasized the importance of psychological factors in the evaluation of QoL as a primary outcome.[21] Specifically, self-esteem has a significant impact on OHRQoL in adolescents.[22,23]

In a longitudinal study by Benson et al., it was reported that adolescents with higher self-esteem experience the negative effects of their malocclusion less frequently, and any subsequent improvement in OHRQoL is linked to an increase in self-esteem.[24] Thus, adolescents with both severe malocclusion and low self-esteem tend to express higher levels of concern regarding their appearance.[25,26] Therefore, orthodontic treatment may play an important role in improving self-esteem.[24] Nevertheless, research has also shown that improving facial or dental aesthetics does not necessarily lead to higher self-esteem.[26,27]

The present study aims to bridge the gap in understanding the nuanced relationships between orthodontic

treatment needs and their psychosocial impact, specifically targeting self-esteem, self-perception of dentofacial aesthetics and OHRQoL among adolescents in relation to the severity of malocclusion.

Material and methods

The University of Naples Federico II Institutional Research Committee's ethical standards, as well as the 1964 Declaration of Helsinki and its later amendments, were followed in all procedures involving human participants in this study. The study was approved by the Ethics Committee of University of Naples Federico II, Italy (protocol No. 146/2023).

The study was conducted on a sample of consecutive patients recruited from individuals who attended the Section of Orthodontics of the University of Naples Federico II for the first consultation between September 2022 and June 2023. The inclusion criteria were as follows: healthy individuals aged between 10 and 18 years; ability to correctly understand the questionnaires; and willingness of the patients and their parents to participate in the study. Patients undergoing orthodontic treatment or those with a history of previous orthodontic treatment, maxillofacial surgery and facial trauma, as well as individuals with genetic syndromes, craniofacial alterations and neurological diseases were excluded.

Data collection

Three questionnaires were administered to all patients who met the inclusion criteria using an online form before they were seated in the dental chair.

The Psychosocial Impact of Dental Aesthetics Questionnaire (PIDAQ) contains 23 questions distributed across 4 domains: Dental Self-Confidence (DSC) (6 items); Social impact (SI) (8 items); Psychological Impact (PI) (6 items); and Aesthetic Concern (AC) (3 items).[28] For this study, the Italian version of the PIDAQ was used, adapted for adolescents and composed of 23 items distributed across 3 domains: AC (4 items); Psychosocial Impact (PSI) (13 items); and DSC (6 items).[29] The total score of the questionnaire is calculated by summing the scores for all items, measured on a Likert scale from 0 to 4, as follows: 0 – "not at all"; 1 – "a little"; 2 – "moderate"; 3 – "severe"; and 4 – "very severe". All items in the DSC domain that were formulated positively were scored inversely, bringing the direction of the score in line with the other subscales to facilitate the interpretation of the results and allow the total score to be calculated.[28]

The Orofacial Esthetic Scale (OES) is composed of 8 items, of which the first seven refer to the appearance of the face, mouth, teeth, and potential prosthetic replacements, and the 8th is an element of global evaluation. An 11-point numerical scale is used for each item (from 0 – "very dissatisfied" to 10 – "very satisfied"). The first seven items are combined into a summary score ranging from 0 to 70, while the 8th item provides an overall response regarding the patient's global assessment of orofacial aesthetics and is not included in the total score.[30] The translated and validated Italian version of the OES was used in this study.[31]

The Rosenberg Self-Esteem Scale (RSES) is a 10-item instrument designed to measure an individual's perception of self-esteem. The questionnaire comprises 5 positively worded items and 5 negatively worded items.[32] The Likert response scale ranges from 1 to 4 (1 – "strongly disagree"; 2 – "disagree"; 3 – "agree"; and 4 – "strongly agree"). The scoring is reversed for negatively worded items. The total score ranges from 10 to 40 points, and the sum of the scores provides an estimation of self-esteem. Scores below 15 suggest low self-esteem, those ranging between 15 and 25 indicate moderate self-esteem, and scores above 26 suggest high self-esteem. The translated and validated Italian version of the RSES was used in this study.[33]

A comprehensive medical and dental history, functional assessment, extraoral and intraoral photographs, an orthopantomogram, lateral cephalometric radiographs, and digital impressions were all part of the standardized first orthodontic visit. The collected clinical data was used to evaluate OVB, OVJ and the need for orthodontic treatment. The measurement of OVB and OVJ was conducted directly by 2 trained dentists on digital models. Overjet and OVB were categorized into 3 distinct groups: reduced; normal; and increased. The normal range for both OVJ and OVB was defined as 1–3 mm. The need for orthodontic treatment was evaluated using the index of orthodontic treatment need (IOTN).[34] The IOTN has 2 separate components that are recorded independently: the Dental Health Component (DHC); and the Aesthetic Component (AC). The IOTN-DHC assesses the orthodontic treatment need in 5 categories, ranging from 1 (treatment is not necessary) to 5 (high treatment need), and taking into account multiple components of occlusion. The IOTN-AC evaluates the patient's subjective dental situation using a series of intraoral photographs numbered from 1 to 10, which are shown to the patient. The patient is then asked to select the photograph that best reflects their smile. In order to facilitate comparison between self-perceived and objective malocclusion severity, the sample was divided into 3 groups according to orthodontic treatment need: no need/ little need (IOTN-DHC 1–2/ IOTN-AC 1–4); moderate need (IOTN-DHC 3/IOTN-AC 5–7); and definite need (IOTN-DHC 4–5/ IOTN-AC 8–10).

The study also included a variety of orthodontic parameters obtained from cephalometric analysis, which assessed the anteroposterior and vertical relationships of the maxilla and mandible with the cranial base and with each other. The following parameters were measured: the sella–nasion–A point angle (SNA) (normal value: 82° ±3.5°); the sella–nasion–pogonion angle (SNPg) (normal value:

80° ±3.5°); the A point–nasion–pogonion angle (ANPg) (normal value: 2° ±2.5°); the angle between the sella–nasion plane and the anterior nasal spine–posterior nasal spine plane (SN/ANSPNS) (normal value: 8° ±3°); the angle between the sella–nasion plane and the gonion–gnathion mandibular plane (SN/GoGn) (normal value: 33° ±2.5°); and the angle between the palatal plane and the mandibular plane (ANSPNS/GoGn) (normal value: 25° ±6°). These parameters were selected because they comprehensively describe anteroposterior discrepancies (SNA, SNPg, ANPg) and vertical growth tendencies (SN/ANSPNS, SN/GoGn, ANSPNS/GoGn), both of which influence facial aesthetics and, consequently, self-perception. The parameters were divided into 3 categories, namely increased, normal and reduced. A description of these parameters and the classification of the subgroups are provided in the Supplementary Material (Supplementary Table 1 (available on request from the corresponding author)).

Statistical analysis

Categorical variables were reported as frequencies and percentages, whereas continuous variables were presented as means (*M*) and standard deviations (*SD*s) when normally distributed or as medians (*Me*s) and interquartile ranges (*IQR*s) when the data showed a skewed distribution. The Shapiro–Wilk test was used to assess normality.

The analysis of continuous variables and comparisons between groups (more than 2 for categorical variables) were performed using analysis of variance (ANOVA) or the Kruskal–Wallis test, depending on whether the continuous variable followed a normal or non-normal distribution. When comparisons were made between categorical variables, the χ^2 test was used.

Correlations between continuous variables were assessed by calculating Pearson's *r* or Spearman's rho, depending on whether both variables were symmetrical or whether at least 1 variable was asymmetrical. Scatter plots were used to present the data graphically.

The association between a specific continuous variable (dependent variable) and the results of a psychological questionnaire was evaluated using univariable and multivariable linear regression models. Beta coefficients and 95% confidence intervals (*CI*s) were calculated for all models.

A p-value <0.05 was considered statistically significant. All analyses were performed using Stata 18 (StataCorp LLC, College Station, USA).

Results

The study included a total of 196 adolescents, comprising 85 males and 111 females. The mean age of the participants was 13.01 ±2.39 years. The values for the questionnaires used in the study, expressed as *Me* (*IQR*), were as follows: PIDAQ-DSC – 18.0 (13.0, 22.0); PIDAQ-PSI – 13.0 (6.0, 24.0); PIDAQ-AC – 6.0 (3.0, 8.0); PIDAQ total – 37.0 (24.5, 52.0); the Global Self-Assessment item of the OES – 6.0 (5.0, 8.0); OES total – 41.0 (31.0, 48.0). The *M* ±*SD* value for the RSES was 29.8 ±5.2.

The descriptive statistics of the orthodontic parameters and IOTN scores are shown in Table 1 and Table 2. More than half of the adolescent patients (58.2%) were classified as having a definite need for orthodontic treatment according to the clinician's objective IOTN-DHC evaluation. However, the majority of these patients (64.3%) rated their own need for orthodontic treatment as non-existent of low according to the subjective IOTN-AC assessment. Most adolescent patients exhibited normal values of SNA (63.8%), SNPg (60.2%), ANPg (59.2%), SN/ANSPNS (68.9%), and ANSPNS/GoGn (76.5%). However, for SN/GoGn, the number of patients with decreased (33.7%), normal (36.7%) and increased (29.6%) values was nearly equal. Additionally, a substantial proportion of the adolescent patients exhibited increased OVJ (50.5%) and OVB (42.3%) values.

The results of the PIDAQ, OES and RSES in relation to the IOTN-DHC groups are presented in Table 3.

Table 1. Descriptive statistics of orthodontic and cephalometric parameters in the study population

Variable	Result	Reduced, *n* (%)	Normal, *n* (%)	Increased, *n* (%)
OVJ	3.5 (2.0, 5.0)	19 (9.7)	78 (39.8)	99 (50.5)
OVB	3.0 (1.0, 4.4)	38 (19.4)	75 (38.3)	83 (42.3)
SNA	81.5 (4.0)	39 (19.9)	125 (63.8)	32 (16.3)
SNPg	78.7 (3.9)	56 (28.6)	118 (60.2)	22 (11.2)
ANPg	3.0 (0.9, 4.8)	24 (12.2)	116 (59.2)	56 (28.6)
SN/ANSPNS	8.7 (3.2)	26 (13.3)	135 (68.9)	35 (17.9)
SN/GoGn	33.0 (5.5)	66 (33.7)	72 (36.7)	58 (29.6)
ANSPNS/GoGn	24.4 (5.1)	17 (8.7)	150 (76.5)	29 (14.8)

Non-normally distributed variables are presented as median (*Me*) (interquartile range (*IQR*), while normally distributed variables are presented as mean (*M*) (standard deviation (*SD*)).
OVJ – overjet; OVB – overbite; SNA – sella–nasion–A point angle; SNPg – sella–nasion–pogonion angle; ANPg – A point–nasion–pogonion angle; SN/ANSPNS – angle between the sella–nasion plane and the anterior nasal spine–posterior nasal spine plane; SN/GoGn – angle between the sella–nasion plane and the gonion–gnathion mandibular plane; ANSPNS/GoGn – angle between the palatal plane and the mandibular plane.

Table 2. Distribution of the index of orthodontic treatment need (IOTN) components in the study population

Component	Result *Me* (*IQR*)	No/little need, *n* (%)	Moderate need, *n* (%)	Definite need, *n* (%)
IOTN-DHC	4.0 (3.0, 4.0)	31 (15.8)	51 (26.0)	114 (58.2)
IOTN-AC	4.0 (2.0, 6.0)	126 (64.3)	38 (19.4)	32 (16.3)

DHC – Dental Health Component; AC – Aesthetic Component.

Table 3. Comparison of subjective assessment with the objective measurement of orthodontic treatment need

Questionnaire	Subgroup/domain	IOTN-DHC			*p*-value
		no/little need	moderate need	definite need	
IOTN-AC, *n* (%)	no/little need	21 (67.7)	34 (66.7)	71 (62.3)	0.725
	moderate need	7 (22.6)	10 (19.6)	21 (18.4)	
	definite need	3 (9.7)	7 (13.7)	22 (19.3)	
PIDAQ *Me* (*IQR*)	DSC	17.0 (13.0, 21.0)	18.0 (13.0, 21.0)	18.5 (13.0, 22.0)	0.333
	PSI	15.0 (4.0, 22.0)	12.0 (7.0, 23.0)	15.0 (6.0, 24.0)	0.663
	AC	6.0 (4.0, 9.0)	6.0 (2.0, 8.0)	6.0 (3.0, 8.0)	0.797
	total	38.0 (22.0, 48.0)	34.0 (25.0, 52.0)	38.0 (25.0, 53.0)	0.738
OES *Me* (*IQR*)	global self-assessment	6.0 (5.0, 8.0)	6.0 (5.0, 8.0)	6.5 (5.0, 8.0)	0.851
	total	41.0 (29.0, 49.0)	43.0 (35.0, 48.0)	38.5 (29.0, 48.0)	0.354
RSES *M* (*SD*)		30.4 (5.2)	29.7 (4.9)	29.7 (5.4)	0.802

PIDAQ – Psychosocial Impact of Dental Aesthetics Questionnaire; DSC – Dental Self-Confidence; PSI – Psychosocial Impact; AC – Aesthetic Concern; OES – Orofacial Esthetic Scale; RSES – Rosenberg Self-Esteem Scale.

No statistically significant differences were found between the IOTN-AC groups and the subdomains of the PIDAQ, OES and RSES, with respect to the IOTN-DHC groups. The overall and subdomain median scores of the PIDAQ and OES, as well as the mean RSES scores, showed minimal variation across the different IOTN-DHC subgroups.

Table 4 presents the results for the IOTN-AC subgroups, PIDAQ and OES subdomains, and RSES according to the subgroups of OVJ, OVB, and skeletal malocclusion.

There was a statistically significant difference between the RSES and OVJ ($p = 0.042$), with RSES scores increasing as OVJ values increased. Additionally, significant differences were identified between the OVB subgroups with respect to the PIDAQ-DSC ($p = 0.040$) and the Global Self-Assessment item of the OES ($p = 0.028$). The PIDAQ-DSC scores decreased with increasing OVJ values, whereas the Global Self-Assessment scores of the OES were lower in the normal OVJ group and higher in the increased OVJ group compared to the decreased OVJ group. Furthermore, a statistically significant difference was observed between the IOTN-AC subgroups and SN/ANSPNS. No statistically significant differences were identified between the orthodontic parameters SNA, ANPg, SNPg, SN/GoGn and ANSPNS/GoGn and the IOTN-AC subgroups, PIDAQ and OES subdomain and total scores, or RSES total scores.

The correlations between the subdomains and the total scores of the PIDAQ, OES, RSES, and IOTN-AC are shown in Table 5. All PIDAQ subdomain scores and the total PIDAQ score were positively correlated with the overall IOTN-AC scores, with correlation coefficients ranging from 0.149 to 0.313, and all correlations being statistically significant. A weak negative correlation was observed between the OES total score and the IOTN-AC, PIDAQ-DSC, PIDAQ-PSI, PIDAQ-AC, and total PIDAQ scores. However, no statistically significant differences were detected. The total RSES score demonstrated a negative correlation with all PIDAQ subdomain scores and the total PIDAQ score ($p < 0.001$). Additionally, a negative correlation was observed between the total RSES score and the IOTN-AC, although this association did not reach statistical significance. Furthermore, a positive but not statistically significant correlation was identified between the OES and the RSES. The scatter plot illustrating these correlations can be found in the Supplementary Materials (Supplementary Fig. 1).

A regression analysis was conducted to investigate whether the relationship between the total scores of the PIDAQ and IOTN-DHC was influenced by the RSES. The univariate model showed that there was no association between the IOTN-DHC and PIDAQ (no/little need vs. moderate need B = 0.13, 95% *CI*: −0.56–0.83, $p = 0.789$; no/little need vs. definite need B = 0.32, 95% *CI*: −0.29–0.94, $p = 0.312$). The multivariable model revealed that the RSES did not influence the relationship between the PIDAQ and IOTN-DHC (no/little need vs. moderate need B = 0.031, 95% *CI*: −0.548–0.611, $p = 0.915$; no/little need vs. definite need B = 0.205, 95% *CI*: −0.311–0.721, $p = 0.434$). These results suggest that the RSES did not have a significant influence on the relationships between these variables.

Table 4. Comparison of subjective assessment with overjet (OVJ), overbite (OVB) and cephalometric parameter categories

Variable		IOTN-AC, *n* (%)			PIDAQ *Me* (*IQR*)				OES *Me* (*IQR*)		RSES *M* (*SD*)
		no/little need	moderate need	definite need	DSC	PSI	AC	total	global self-assessment	total	
OVJ	R	13 (68.4)	4 (21.1)	2 (10.5)	20.0 (16.0, 23.0)	18.0 (7.0, 27.0)	6.0 (2.0, 12.0)	41.0 (28.0, 54.0)	7.0 (6.0, 8.0)	45.0 (33.0, 49.0)	27.5 (6.7)
	N	53 (67.9)	13 (16.7)	12 (15.4)	18.0 (14.0, 21.0)	13.5 (5.0, 24.0)	6.0 (3.0, 8.0)	38.0 (25.0, 51.0)	6.0 (5.0, 7.0)	40.5 (33.0, 47.0)	29.3 (4.5)
	I	60 (60.6)	21 (21.2)	18 (18.2)	17.0 (12.0, 22.0)	13.0 (6.0, 24.0)	5.0 (3.0, 8.0)	37.0 (23.0, 52.0)	6.0 (5.0, 8.0)	40.0 (28.0, 49.0)	30.6 (5.3)
p-value			0.829		0.193	0.856	0.855	0.604	0.326	0.699	0.042*
OVB	R	22 (57.9)	9 (23.7)	7 (18.4)	20.0 (16.0, 22.0)	18.0 (8.0, 33.0)	6.0 (3.0, 10.0)	42.0 (28.0, 63.0)	6.0 (5.0, 8.0)	42.0 (33.0, 49.0)	28.3 (5.6)
	N	49 (65.3)	17 (22.7)	9 (12.0)	18.0 (13.0, 21.0)	12.0 (6.0, 26.0)	6.0 (3.0, 8.0)	38.0 (26.0, 53.0)	7.0 (6.0, 8.0)	42.0 (35.0, 49.0)	29.9 (5.1)
	I	55 (66.3)	12 (14.5)	16 (19.3)	17.0 (12.0, 21.0)	13.0 (5.0, 22.0)	5.0 (3.0, 8.0)	35.0 (22.0, 51.0)	6.0 (4.0, 7.0)	38.0 (27.0, 46.0)	30.4 (5.1)
p-value			0.471		0.040*	0.238	0.452	0.107	0.028*	0.092	0.123
SNA	R	24 (61.5)	7 (17.9)	8 (20.5)	18.0 (12.0, 21.0)	9.0 (6.0, 21.0)	4.0 (2.0, 7.0)	33.0 (23.0, 49.0)	7.0 (5.0, 8.0)	41.0 (33.0, 52.0)	30.2 (4.8)
	N	81 (64.8)	25 (20.0)	19 (15.2)	18.0 (14.0, 22.0)	15.0 (6.0, 24.0)	6.0 (3.0, 8.0)	38.0 (26.0, 53.0)	6.0 (5.0, 8.0)	41.0 (30.0, 47.0)	29.7 (5.2)
	I	21 (65.6)	6 (18.8)	5 (15.6)	17.0 (12.0, 22.0)	11.0 (5.5, 24.0)	5.5 (4.0, 8.5)	34.0 (22.5, 51.5)	6.0 (5.0, 8.0)	39.5 (29.0, 48.0)	29.8 (5.9)
p-value			0.968		0.822	0.677	0.192	0.485	0.669	0.611	0.858
SNPg	R	33 (58.9)	9 (16.1)	14 (25.0)	18.0 (13.5, 20.5)	14.0 (5.5, 23.0)	6.0 (4.0, 8.0)	38.0 (25.0, 50.5)	7.0 (6.0, 8.0)	41.5 (32.0, 49.0)	30.2 (5.1)
	N	80 (67.8)	24 (20.3)	14 (11.9)	18.0 (13.0, 21.0)	12.5 (6.0, 24.0)	6.0 (3.0, 8.0)	36.5 (23.0, 53.0)	6.0 (5.0, 8.0)	39.5 (29.0, 46.0)	29.7 (5.1)
	I	13 (59.1)	5 (22.7)	4 (18.2)	22.0 (16.0, 22.0)	15.0 (7.0, 27.0)	6.5 (2.0, 9.0)	39.0 (30.0, 52.0)	6.5 (6.0, 8.0)	44.0 (33.0, 53.0)	29.1 (6.3)
p-value			0.279		0.138	0.727	0.857	0.562	0.194	0.285	0.689
ANPg	R	18 (69.2)	4 (15.4)	4 (15.4)	18.0 (12.0, 24.0)	13.5 (5.0, 26.0)	6.0 (4.0, 8.0)	37.5 (22.0, 58.0)	6.0 (5.0, 8.0)	40.5 (27.0, 52.0)	30.0 (6.0)
	N	72 (62.1)	26 (22.4)	18 (15.5)	18.0 (14.0, 22.0)	14.5 (6.0, 24.0)	6.0 (3.0, 8.0)	38.0 (25.5, 53.0)	7.0 (5.0, 8.0)	41.0 (32.0, 48.0)	29.7 (5.0)
	I	36 (64.3)	9 (16.1)	11 (19.6)	17.0 (12.0, 20.5)	13.0 (6.0, 23.0)	6.0 (4.0, 8.0)	37.0 (23.0, 51.0)	6.0 (5.0, 7.0)	38.5 (29.0, 45.5)	29.8 (5.5)
p-value			0.633		0.434	0.711	0.618	0.746	0.087	0.076	0.938
SN/ANSPNS	R	18 (69.2)	4 (15.4)	4 (15.4)	18.0 (12.0, 24.0)	13.5 (5.0, 26.0)	6.0 (4.0, 8.0)	37.5 (22.0, 58.0)	6.0 (5.0, 8.0)	40.5 (27.0, 52.0)	30.0 (6.0)
	N	94 (69.6)	23 (17.0)	18 (13.3)	18.0 (13.0, 22.0)	12.0 (6.0, 23.0)	6.0 (3.0, 8.0)	37.0 (25.0, 52.0)	6.0 (5.0, 8.0)	40.0 (29.0, 48.0)	29.7 (5.2)
	I	14 (40.0)	11 (31.4)	10 (28.6)	18.0 (13.0, 22.0)	18.0 (6.0, 26.0)	5.0 (2.0, 9.0)	39.0 (23.0, 51.0)	7.0 (6.0, 8.0)	44.0 (34.0, 48.0)	29.9 (4.9)
p-value			0.026*		0.839	0.788	0.873	0.927	0.274	0.363	0.975
SN/GoGn	R	47 (71.2)	12 (18.2)	7 (10.6)	17.0 (12.0, 21.0)	14.5 (5.0, 23.0)	5.5 (2.0, 7.0)	37.0 (22.0, 51.0)	6.0 (5.0, 8.0)	40.5 (27.0, 46.0)	29.5 (5.7)
	N	43 (59.7)	14 (19.4)	15 (20.8)	18.0 (13.0, 22.0)	11.5 (6.0, 24.0)	6.0 (3.5, 8.0)	35.5 (26.0, 51.0)	6.5 (5.0, 8.0)	40.5 (31.0, 48.0)	30.0 (5.2)
	I	36 (62.1)	12 (20.7)	10 (17.2)	18.0 (15.0, 22.0)	14.5 (7.0, 24.0)	6.0 (3.0, 9.0)	38.5 (24.0, 54.0)	7.0 (5.0, 8.0)	42.0 (33.0, 49.0)	29.8 (4.7)
p-value			0.549		0.626	0.815	0.366	0.698	0.586	0.463	0.891
ANSPNS/GoGn	R	19 (65.5)	7 (24.1)	3 (10.3)	21.0 (17.0, 23.0)	16.0 (9.0, 24.0)	6.0 (5.0, 7.0)	40.0 (34.0, 51.0)	6.0 (5.0, 7.0)	38.0 (30.0, 45.0)	28.0 (4.6)
	N	93 (62.0)	29 (19.3)	28 (18.7)	18.0 (13.0, 22.0)	12.5 (6.0, 24.0)	6.5 (5.0, 8.0)	37.0 (24.0, 52.0)	6.5 (5.0, 8.0)	41.0 (31.0, 48.0)	30.2 (5.2)
	I	14 (82.4)	2 (11.8)	1 (5.9)	19.0 (12.0, 22.0)	11.0 (6.0, 22.0)	7.0 (5.0, 9.0)	35.0 (23.0, 52.0)	7.0 (5.0, 9.0)	43.0 (33.0, 52.0)	29.5 (5.7)
p-value			0.390		0.119	0.634	0.303	0.485	0.301	0.339	0.123

R – reduced; N – normal; I – increased; * statistically significant ($p < 0.05$). Comparisons were made using the χ^2 test (IOTN-AC), the Kruskal–Wallis test (PIDAQ, OES) and one-way ANOVA (RSES).

Table 5. Correlation between Psychosocial Impact of Dental Aesthetics Questionnaire (PIDAQ) subdomain and total scores, Orofacial Esthetic Scale (OES) total score, Rosenberg Self-Esteem Scale (RSES) total score and the Aesthetic Component of the index of orthodontic treatment need (IOTN-AC) score

Questionnaire	IOTN-AC	PIDAQ-DSC	PIDAQ-PSI	PIDAQ-AC	PIDAQ total	OES total
PIDAQ-DSC	0.281, <0.001*	–	–	–	–	–
PIDAQ-PSI	0.313, <0.001*	–	–	–	–	–
PIDAQ-AC	0.149, 0.036	–	–	–	–	–
PIDAQ total	0.312, <0.001*	–	–	–	–	–
OES total	−0.012, 0.857	−0.038, 0.590	−0.116, 0.102	−0.017, 0.807	−0.091, 0.199	–
RSES	−0.123, 0.084	−0.452, <0.001*	−0.578, <0.001*	−0.187, <0.001*	−0.5575, <0.001*	0.139, 0.051

Data expressed as *r* values followed by *p*-values; * statistically significant ($p < 0.05$, Spearman's rho).

Discussion

The objective of this study was to investigate the intricate relationship between malocclusion severity and adolescents' self-perception of dentofacial aesthetics, OHRQoL and self-esteem. In this study, adolescents exhibited a lower perceived need for orthodontic treatment compared with the objective assessment of clinicians. Additionally, a significant correlation was identified between the psychosocial impact of dental aesthetics on OHRQoL and subjective orthodontic treatment need, as well as self-esteem.

Among the instruments used in this study, the PIDAQ and RSES are reliable tools that have been successfully used in previous studies involving adolescents.[29,35] Although the OES was developed for adult patients,[30,31] the authors did not consider it necessary to adapt it for adolescents, as it consists of very simple and easily understandable questions. Self-perceived and normative orthodontic treatment need were evaluated using the IOTN-AC and IOTN-DHC, respectively, considering that IOTN is a widely used and validated index.[36,37]

A significant proportion of adolescents classified by clinicians as having a definite need for orthodontic treatment according to the IOTN-DHC perceived their own need for treatment as low or non-existent based on the IOTN-AC. This discrepancy is noteworthy, especially considering that many healthcare systems base patient selection criteria on the IOTN-DHC, which may not accurately reflect adolescents' perceptions of their orthodontic treatment needs. Furthermore, it emphasizes the significance of incorporating patients' subjective evaluations into orthodontic treatment planning in addition to objective assessments. Similar to our findings, Chauhan et al. identified a statistically significant difference between the definite-need categories of the IOTN-DHC and IOTN-AC, with adolescents being less critical in their aesthetic evaluations.[38] Conversely, Borzabadi-Farahani and Borzabadi-Farahani observed a moderate degree of agreement between normative and subjective orthodontic treatment needs, as determined by the IOTN components.[39] The reason for this discrepancy may be that the IOTN-DHC evaluates specific occlusal traits, such as ectopic teeth, hypodontia, deep traumatic bites, or cross-bites, all of which have implications for dental health. However, these conditions do not necessarily correspond to high aesthetic concern. Therefore, IOTN-DHC encompasses a series of parameters that may not be readily understood by patients, particularly adolescents, resulting in these components being assigned less importance. The IOTN-AC evaluates the aesthetic aspects of malocclusion using a two-dimensional frontal view, thereby underscoring its subjective nature.[39] In fact, the reliability of the IOTN-AC remains a matter of debate in the literature.[40–42] In contrast to our findings, dos Santos et al. reported that children who self-declared a need for orthodontic treatment (IOTN-AC) were likewise diagnosed using the normative criterion (IOTN-DHC).[43]

Given the monocentric nature of this study, cultural and societal norms may play a crucial role in shaping self-perception and assessments of orthodontic treatment need. A study by Burden highlighted that an individual's decision to undergo orthodontic treatment is influenced more by their peers' familiarity with orthodontic appliances than by factors such as sex or socioeconomic status.[44]

Although the present study did not directly assess treatment adherence or uptake, the discrepancy between adolescents' perceived and clinician-assessed orthodontic treatment need may affect their motivation and cooperation. Addressing this communication gap is crucial, as effective clinician engagement and clear discussions of treatment objectives may enhance adherence. Moreover, studies have demonstrated that tailored communication strategies play a key role in improving patient compliance.[45,46]

These findings may also explain the absence of significant differences in self-esteem, the psychological impact of dental aesthetics and self-perceived orofacial aesthetics in relation to normative orthodontic treatment need. Since adolescents' perceived orthodontic treatment need appears to be lower than their normative orthodontic need, objective treatment need may not negatively affect these psychosocial outcomes. Consistent with our findings, Gavric et al. reported only modest correlations between the IOTN-DHC and PIDAQ scores.[12] Their results indicated that subjective perceptions of orthodontic treatment need exert a greater influence on OHRQoL than objective orthodontic treatment need.[12] However, these outcomes

contrast with those of Bellot-Arcís et al., who reported a significant linear association between PIDAQ scores and the grades of both IOTN components among Spanish adolescents.[47] The relationship between self-esteem and normative orthodontic treatment need remains controversial. Similar to our findings, Akpasa et al. did not report any significant associations between self-esteem and normative orthodontic treatment need.[48] However, other studies have identified a significant relationship between self-esteem and normative orthodontic treatment need.[12]

In line with the findings of Dallé et al.,[49] there was no relationship between skeletal malocclusion characteristics and OHRQoL in our study. Conversely, Pereira et al. reported that OHRQoL was significantly impacted by the severity of skeletal malocclusion.[17] The lack of a significant correlation between the characteristics of skeletal malocclusion and OHRQoL in this study can be partially attributed to the predominance of normal skeletal cephalometric measurements among adolescent patients. Specifically, 63.8% of participants exhibited normal SNA values, 60.2% demonstrated normal SNPg values, 59.2% presented normal ANPg values, 68.9% had normal SN/ANSPNS values, and 76.5% presented normal ANSPNS/GoGn values. These figures suggest that the majority of the sample did not present with severe skeletal discrepancies, which are more likely to have a pronounced impact on facial aesthetics and psychosocial well-being.

This result may also be due to the reduced aesthetic impact of the facial profile when viewed from the front, which is the perspective from which people typically perceive themselves in daily life.[50] A reduced OVB, indicative of an anterior open bite and more evident in the frontal view, may possibly account for the lower OHRQoL observed in patients with reduced OVB. However, Fabian et al. found a minimal impact of OVB variations on OHRQoL.[14] Additionally, poorer self-esteem was observed in adolescents with reduced OVJ, which may indicate the presence of an inverse (negative) OVJ that can be more evident from the frontal view than a normal or increased OVJ.

The impact of OVJ and OVB on psychosocial well-being is also likely influenced by their high visibility in social interactions. Malocclusions affecting the anterior dentition are more noticeable and can influence self-perception, peer interactions and social acceptance, thereby affecting self-esteem and OHRQoL.[14,51] Additionally, individuals with severe deviations in OVJ or OVB may experience functional difficulties, such as speech alterations or difficulty biting,[52] which may further contribute to psychological distress.

In line with previous research,[24,53] our findings showed that adolescents who perceived a greater need for orthodontic treatment also reported lower OHRQoL related to the psychosocial impact of dental aesthetics. Therefore, irrespective of the objective severity of malocclusion, an individual's subjective assessment of their dental aesthetics may negative affect their OHRQoL.[49] Furthermore, the current research demonstrated a relationship between the psychosocial impact of dental aesthetics on OHRQoL and self-esteem, with adolescents reporting poorer OHRQoL as their self-esteem decreased. This outcome is consistent with previous reports in the literature,[22,54,55] and this relationship may be explained by the vital role of self-esteem in mediating the effects of psychological and physical conditions, as it can act as a protective factor against the adverse effects of various conditions.[23,56,57] Therefore, high levels of self-esteem in adolescents may protect them from the negative effects of malocclusion on their OHRQoL.

One of the objectives of the study was to examine whether self-esteem moderates the association between normative orthodontic treatment need and OHRQoL. The results of previous studies are consistent with our finding, indicating that self-esteem does not exert a moderating effect on the relationship between OHRQoL and normative orthodontic treatment need.[54,58,59] However, Herkrath et al. reported that self-esteem had a moderating influence on the association between OHRQoL and the severity of malocclusion in the aesthetic zone.[59]

Only a limited number of studies have used the OES in adolescent populations. A study by Kovacevic Pavicic et al. involving young adults reported no association between the PIDAQ and OES scores,[60] which is consistent with the findings of this study. Further research is required to investigate the relationship between self-perceived orofacial appearance, normative and subjective orthodontic treatment need and self-esteem. Additionally, longitudinal studies assessing changes in self-esteem and OHRQoL before and after treatment would provide valuable insights.

As prior research has mainly evaluated the dental components of malocclusion, one of the strengths of the present study was the evaluation of the relationship between self-esteem, OHRQoL, and self-perception of malocclusion and dentofacial aesthetics with skeletal malocclusions using cephalometric parameters that assessed maxillary and mandibular position in both the sagittal and vertical planes.

A limitation of this study was the absence of an evaluation of the socioeconomic status of the patients, which could have influenced their awareness of their oral condition or the perceived need for orthodontic care. Furthermore, the study did not address potential sex differences in the perception of dental aesthetics, despite evidence suggesting that aesthetic concerns may vary between males and females.[61,62] It is recommended that future studies include socioeconomic status and sex as variables to provide a more comprehensive understanding of their impact on orthodontic treatment perceptions and outcomes.

Although the study included adolescents aged 10–18 years, the analysis did not divide participants into younger (10–13 years) and older (14–18 years) groups to investigate potential differences in psychosocial outcomes. This approach was chosen to maintain a sufficient sample size for statistical power. However, the authors

acknowledge that differences between these age groups could provide valuable insights, as younger and older adolescents may face unique psychosocial challenges and self-esteem dynamics. To address this limitation, future research should consider stratifying participants by age group to better capture these distinctions and their clinical relevance.

As this was a monocentric study involving adolescents seeking orthodontic treatment, the results may not be representative of other geographic regions with different cultural and social backgrounds, and may not fully reflect perceptions of oral health in the general population. To reduce potential selection bias, future research should include more diverse samples comprising adolescents from various settings, including those who are not actively seeking orthodontic treatment.

Another limitation of the present study is the absence of a pilot study evaluating the applicability of the OES in an adolescent population. Although the questionnaire was originally developed for adults and no comprehension difficulties were reported by participants, the authors recommend that future research include a formal validation of the OES specifically for adolescent patients.

The findings of this study highlight the importance of clearly communicating treatment objectives to adolescent patients while offering alternative options to encourage their engagement throughout the treatment process. Shared decision-making may facilitate this process by actively involving patients and their families in treatment planning. Furthermore, the use of digital visual aids and patient-reported outcome measures may help align clinical recommendations with patients' expectations and concerns.

Additionally, given that OVJ and OVB were identified as key clinical factors associated with the psychological impact of dental aesthetics, self-esteem and self-perceived orofacial aesthetics, an early intervention approach aimed at addressing these occlusal discrepancies may be beneficial in mitigating their psychosocial consequences.

Conclusions

The use of objective indices, such as the IOTN-DHC, to assess orthodontic treatment need does not correspond closely to adolescents' self-perceived need for treatment. Therefore, before initiating orthodontic treatment, it is important to understand patients' expectations and treatment objectives, as they may not always coincide with those of the orthodontist. Clinicians may employ shared decision-making approaches, including patient-reported outcome measures and digital visual aids, to help address this discrepancy.

Oral health-related quality of life is associated with adolescents' self-esteem and their perceived need for orthodontic treatment, but not with skeletal malocclusion characteristics. Furthermore, addressing psychosocial factors during orthodontic consultations is particularly important in adolescents with significant OVJ or OVB deviations, as these malocclusion traits may substantially influence the psychological impact of dental aesthetics, self-esteem and self-perceived orofacial aesthetics.

Ethics approval and consent to participate

This study followed ethical standards and guidelines for research involving human participants, including the 1964 Helsinki Declaration and its later amendments. The research was approved by the Ethics Committee of the University of Naples Federico II, Italy (protocol No. 146/2023). Written informed consent was obtained from all study 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

Skill acquisition in Dentistry degree during the COVID-19 pandemic in Spain

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Abstract

Background. The acquisition of dental skills during the final year of the Dentistry degree is primarily based on clinical training, which was severely disrupted during the coronavirus disease 2019 (COVID-19) pandemic.

Objectives. The aim of the study was to determine the degree of acquisition of specific skills in the Integrated Dental Clinic course based on students' perceptions.

Material and methods. A total of 84 dental students were included in the study. Online forms were administered to evaluate students' perception of competency acquisition in professionalism, communication, basic knowledge, information management, critical thinking, collection of clinical information, diagnosis, treatment, health establishment, health maintenance, and health promotion. The questionnaires were sent before and after the declaration of the COVID-19 state of alarm in Spain. Statistical analysis was performed using R statistical software, v. 4.1.1.

Results. The highest percentage of students reporting high levels of perceived competency acquisition corresponded to health promotion (72.37%), professionalism (52.86%) and ability to collect clinical information (52.34%). Statistically significant improvements in perceived skill acquisition between the beginning and the end of the course were observed for professionalism, collection of clinical information, diagnosis, treatment, health establishment, health maintenance, and health promotion.

Conclusions. Despite the disruptions caused by the pandemic, fifth-year dental students perceived that they had successfully acquired specific skills by the end of the academic course, particularly professional skills, ability to collect clinical information, ability to diagnose and treat, ability to establish and maintain health, as well as health promotion skills.

Keywords: COVID-19, resources, competencies, skills in dentistry, dental education

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Highlights

- The study demonstrates that rapid adaptation maintained the quality of clinical dental education during the coronavirus disease 2019 (COVID-19) pandemic.
- Students reported significant improvements in skill acquisition, particularly in terms of professionalism, clinical information collection, diagnosis, treatment, and health promotion throughout the academic course.
- Flexible clinical teaching strategies supported continuity of dental education under unprecedented public health challenges.

Introduction

Contemporary models of university education propose the implementation of curricula focused on competency-based education.[1,2] The coronavirus disease 2019 (COVID-19) pandemic has prompted an unexpected methodological shift toward online teaching and learning. Within a short period, educators adapted their teaching practices by incorporating digital technologies, regardless of their professional background. This situation has been described as emergency distance education (EDE), characterized by the intentional adoption of educational technologies.[3] Consequently, experience with information and communication technologies (ICT) and the potential for innovation became relevant, broadening the scope of the teaching role.[4] This transition required educators to adapt creatively to technology-enhanced learning.[5,6] Teachers' expertise in instructional design and course organization is necessary for educational objectives to be fulfilled, supported by feedback, instruction and evaluation.[6]

This transition is limited by the heterogeneous backgrounds of higher-level education professors, whose experience with distance education varies and who often require personalized technological, institutional and pedagogical support.[4] Consequently, the evaluation of competencies has also undergone changes.[7,8] Successful implementation of these changes depended heavily on institutional support.[9] However, some authors have argued that insufficient institutional commitment limits the design, implementation and maintenance of online teaching.[10] Additional challenges included the rapid development of online learning materials and the provision of adequate technical infrastructure and pedagogical support during the emergency transition.[11] The use of digital technologies in these extreme situations can play a catalytic role in the development of educational innovations.

In Spain, the final year of the Dentistry degree is centered on clinical training, which serves as the prelude to the student's incursion into professional life. The curriculum includes an annual course worth 24 European Credit Transfer System and Accumulation System (ECTS) credits, and it is taught together with the courses of Maxillofacial Surgery (6 ECTS), Preventive and Community Dentistry (6 ECTS), 2 elective subjects (12 ECTS), Legal and Forensic Dentistry (6 ECTS), and a Final Degree Project (6 ECTS). The practicum provides supervised training in a real clinical environment, enabling students to acquire professional competencies through experiential learning. Assessment is based on the acquisition of competencies that encompass professional values, communication skills, individualized clinical reasoning and judgment skills, as well as clinical management skills. These supervised activities allow the acquisition of a competent professional profile with appropriate knowledge and skills expected of a competent dentist.[12] Technology-based learning has become an increasingly accepted component of dental education, and its integration into the dental curriculum is considered essential for improving teaching quality.[13] Nevertheless, translating face-to-face clinical dental training into an online learning environment remains a major challenge for educators and has required the rapid development of innovative teaching strategies. Competency-based clinical education in this context represents a substantial challenge for clinical professors.[14] Competencies extend beyond the acquisition of theoretical knowledge, encompassing the skills, attitudes and psychological resources required to address complex healthcare and social needs.[15] The success and high professional standards of dental graduates depend largely on the acquisition of these competencies, which are established as learning objectives by the community directives governing dental education.

Taking this scenario into account, the COVID-19 pandemic raised important concerns regarding dental education. The interruption of clinical patient care and the suspension of many research activities generated uncertainty about the professional preparation of dental students, as the inability to participate in face-to-face clinical training could adversely affect the acquisition of specific skills. Accordingly, the objectives of the present study were to assess students' perceptions of their acquisition of specific skills in the Integrated Dental Clinic course during the pandemic and to determine their satisfaction with the education provided. These findings may inform future improvements in teaching methodologies and support the development of educational strategies that enhance the acquisition of competencies.

Material and methods

The present study was reviewed and approved by the Ethics Committee of the Quality Office of the Complutense University of Madrid, Spain (code No. 375-2019). The study was conducted in accordance with the principles of the Declaration of Helsinki, the recommendations of the World Association of Medical Editors (WAME), the International Committee of Medical Journal Editors (ICMJE) guidelines for the reporting and elaboration of research, and the Spanish Organic Law 3/2018 of December 5 on the Protection of Personal Data and Guarantee of Digital Rights (LOPD).

The study population consisted of 84 fifth-year dental students enrolled in the Dentistry degree program. The required sample size was calculated using the formula for estimating a proportion in a finite population. Assuming a 95% confidence level, an alpha error of 0.05, a beta error of 0.2, and a bilateral hypothesis, the minimum required sample size was 39 students. All eligible students were invited to participate regardless of sex, ethnicity or type of university access. Students who declined to participate were excluded. This cross-sectional study was conducted in 3 phases:

- Phase 1. Research team coordination. Coordination meetings were held to define and distribute responsibilities among the investigators, and develop data collection instruments;
- Phase 2. Questionnaire development and administration. The target population included all students enrolled in the fifth-year Integrated Dental Clinic course. This annual subject comprises 24 ECTS credits, of which 21 are face-to-face supervised training, while the remaining 3 are dedicated to online learning activities, including clinical case preparation and the final degree project. The course is taught in Spanish and is primarily delivered at the university clinic under the supervision of 53 professors and 1 coordinator. Clinical training focuses on the development of discipline and specific skills through the treatment of patients aged 6 years and older. At this stage of the study, the academic guide for the subject was reviewed to collect all possible information about skills that the students were expected to acquire.[12] These competencies were based on the White

Table 1. Elements of the questionnaire administered to the students, organized by competency domain

Section	Item
Professional Skills (H1)	1. Understand patients without prejudice regarding their origin, race or culture
	2. Establish and maintain a safe working environment and appropriate environmental conditions
	3. Handle, distinguish and select appropriate materials and instruments
	4. Evaluate and apply scientific evidence for the benefit of the patient
	5. Select high-quality, objective, valid, up-to-date, and appropriate treatment options
	6. Respect patients and colleagues regardless of sex, diversity or culture
	7. Recognize inappropriate professional conduct, take appropriate action and support patient when necessary
	8. Apply ethical principles in professional practice
Communication and Personal Skills (H2)	9. Establish an effective dentist–patient relationship
	10. Identify the patient's goals and expectations
	11. Identify psychological and social factors associated with oral dysfunction and diagnose, treat or refer the patient as appropriate
	12. Communicate professional information effectively, both verbally and in writing, with patients and other healthcare professionals; discuss issues and give and receive constructive feedback
	13. Apply stress management principles when caring for patients and working with colleagues
	14. Work effectively with other members of the healthcare team
	15. Apply the scientific principles of cleaning, sterilization, disinfection, and asepsis
Basic Knowledge, Information Management and Critical Thinking Skills (H3)	16. Use current electronic communication and information technologies
	17. Assess the validity of claims regarding the benefits of instruments, products and techniques
	18. Evaluate scientific research and clinical studies and integrate this information to improve patient's oral health
Ability to Collect Clinical Information (H4)	19. Identify the patient's chief complaint and obtain the history of the present illness
	20. Obtain a comprehensive medical history
	21. Request a written medical consultation or refer the patient when appropriate
	22. Recognize abnormal patient behavior
	23. Perform a dietary assessment
	24. Perform and evaluate a comprehensive extraoral and intraoral examination, including the recording of vital signs
	25. Take and interpret X-rays and be familiar with other diagnostic techniques
	26. Inform patients about the nature and severity of their diseases, presenting treatment options and realistic expectations
	27. Perform diagnostic procedures in oral pathology, including biopsy, and be familiar with other diagnostic techniques

Section	Item
Ability to Diagnose and Treat (H5)	28. Identify, diagnose and manage prosthetic treatment needs
	29. Diagnose malocclusions
	30. Diagnose medical emergencies
	31. Identify patients with special healthcare needs
	32. Diagnose pathologies described above and develop a prioritized, sequential treatment plan
Ability to Establish and Maintain Health (H6)	33. Apply evidence-based treatments
	34. Provide oral hygiene instructions and apply appropriate preventive and therapeutic measures for each patient
	35. Educate patients about the etiology and prevention of oral diseases and motivate them to take responsibility for their oral health
	36. Provide dietary and nutritional advice to promote oral health
	37. Develop strategies to predict, prevent and correct deficiencies in oral hygiene techniques and monitor adverse oral habits and oral function
	38. Evaluate treatment outcomes and develop an individualized oral health maintenance plan based on patient risk
	39. Perform preventive and restorative procedures that preserve oral structures, prevent diseases and promote oral health
	40. Manage local complications that may occur following surgical treatments
	41. Manage preoperative and postoperative pharmacological treatment
	42. Assess the periodontium and establish a diagnosis, prognosis and periodontal treatment plan
	43. Evaluate periodontal treatment outcomes and implement a maintenance program, including risk factor assessment
	44. Assess the dentition and establish a diagnosis, prognosis and treatment plan
	45. Assess the patient's risk of dental caries and implement individualized preventive strategies
	46. Remove dental caries using techniques that preserve pulp vitality
	47. Diagnose and treat non-carious dental conditions
	48. Perform therapeutic procedures to preserve, establish or restore dental form, vitality, function, and aesthetics
	49. Perform simple root canal treatment and use endodontic instruments appropriately
	50. Recognize when treatment is likely to be complex and take appropriate action
	51. Plan, design and apply adhesive restorations, crowns, simple bridges, and removable prostheses using appropriate materials and appliances
	52. Evaluate, predict and manage simple occlusal disorders and their clinical repercussions
	53. Diagnose the need for orthodontic and orthopedic treatment, understand common treatment techniques, develop a treatment plan, or refer the patient when appropriate
	54. Identify oral habits that may cause or worsen malocclusion and prevent their consequences through education, training or appliances
	55. Design, place and adjust space maintainers and active appliances for less complex treatments
	56. Manage orthodontic emergencies and refer the patient when necessary
	57. Apply local anesthesia techniques for restorative, surgical and other dental procedures
	58. Diagnose and manage orofacial pain and refer the patient when necessary
	59. Select and prescribe medications for the management of pain and anxiety related to dental treatment
	60. Identify the causes and persistence of fear and anxiety associated with dental treatments and manage them using behavioral techniques
	61. Develop and implement effective strategies for the prevention and management of dental and medical emergencies in the dental office
	62. Perform basic cardiopulmonary resuscitation and manage medical emergencies occurring during dental treatment
	63. Identify and manage dental emergencies
	64. Identify unresolved dental or medical emergencies and refer patients promptly
	65. Perform multidisciplinary, sequential, integrated treatments of limited complexity for patients of all ages and clinical conditions
	66. Perform extractions of erupted teeth
	67. Manage dental trauma in both primary and permanent dentitions and be familiar with the surgical and non-surgical aspects of maxillofacial trauma management
	68. Perform uncomplicated surgical extractions of unerupted teeth and fractured or retained roots
	69. Diagnose temporomandibular joint disorders
Health Promotion Skills (H7)	70. Apply the principles of health promotion and disease prevention
	71. Develop and implement health promotion programs and coordinate interinstitutional and interprofessional collaboration

Book of the National Agency for Quality Assessment and Accreditation (ANECA). Data was collected using an online questionnaire created with Google Forms (Google LLC, Mountain View, USA). The questionnaire included sociodemographic variables (sex, age, university admission pathway, and occupation at the time of the survey), as well as 71 questions related to students' perceptions of competency acquisition across 7 sections: Professional Skills; Communication and Personal Skills; Basic Knowledge, Information Management and Critical Thinking Skills; Ability to Collect Clinical Information; Ability to Diagnose and Treat; Ability to Establish and Maintain Health; and Health Promotion Skills (Table 1). Responses were recorded using a 4-point Likert scale, where 1 indicated little or no acquisition of skills and 4 indicated the highest level of skill acquisition. Completion of all questionnaire items was mandatory. To preserve participant anonymity, the questionnaires were distributed by the Dean's Office of the School of Dentistry through e-mails. Anonymous identification codes were assigned to each participant throughout the study. The students received information regarding the study objectives, procedures and planned use of the collected data. Respondents were allowed unlimited time to complete the questionnaire. The initial questionnaire was administered at the beginning of the 2019–2020 academic year, before the declaration of the COVID-19 state of alarm or lockdown in Spain (pre-Q). The follow-up questionnaire was distributed to the students by the Dean's Office in May 2020, at the end of the academic year (post-Q), after the lockdown had been implemented by the government and all teaching activities had transitioned to an online format, with the suspension of face-to-face clinical training. Online teaching was carried out from January 2020 until the end of the second semester in May 2020, coinciding with the government lockdown;

- Phase 3. Data analysis. Statistical analyses were performed using R statistical software, v. 4.1.1. Descriptive analyses were conducted by summarizing students' responses before and after the intervention for each competency domain. Median scores for each item were also taken into consideration and used for the inferential analyses. Differences between the pre-Q and post-Q responses were assessed using the Wilcoxon non-parametric test for paired samples. The results were considered significant for p-values <0.05.

Results

Of the 84 students enrolled, 38 completed both questionnaires (pre-Q and post-Q). The majority of respondents were female (84.4%), and the mean age of the participants was 23.0 years. Most of the students (90.6%) had been admitted to the university through the admission examination, and 28.1% of the participants were employed while studying.

Competency domain analysis

Table 2 presents the distribution of responses for each skill. Significant differences between the beginning and the end of the course were observed for the competency domains of Professional Skills (H1), Ability to Collect Clinical Information (H4), Ability to Diagnose and Treat (H5), Ability to Establish and Maintain Health (H6), and Health Promotion Skills (H7). Figure 1 illustrates the comparison of students' perceived competency acquisition before (pre-Q) and after (post-Q) the COVID-19 lockdown. In most items, students' satisfaction levels increased after lockdown.

Table 2. Distribution of questionnaire responses by competency domain before (pre-Q) and after (post-Q) the coronavirus disease 2019 (COVID-19) lockdown

Likert scale	H1 Pre-Q; Post-Q	H2 Pre-Q; Post-Q	H3 Pre-Q; Post-Q	H4 Pre-Q; Post-Q	H5 Pre-Q; Post-Q	H6 Pre-Q; Post-Q	H7 Pre-Q; Post-Q
4	156 (51); 161 (52)	116 (43); 120 (45)	55 (48); 55 (48)	123 (36); 179 (52)	43 (23); 43 (23)	508 (36); 624 (44)	21 (28); 55 (72)
3	127 (41); 137 (45)	122 (46); 114 (43)	37 (32); 44 (38)	153 (45); 121 (35)	65 (34); 91 (48)	569 (40); 563 (40)	55 (72); 21 (28)
2	21 (7); 6 (2)	28 (11); 32 (12)	22 (19); 15 (13)	61 (18); 42 (12)	82 (43); 56 (29)	297 (21); 219 (16)	0 (0); 0 (0)
1	0 (0); 0 (0)	0 (0); 0 (0)	0 (0); 0 (0)	5 (1); 0 (0)	0 (0); 0 (0)	32 (2); 0 (0)	0 (0); 0 (0)
p-value	0.012*	0.739	0.380	0.000*	0.012*	0.000*	0.000*

Data presented as frequency (percentage) (n (%)); * statistically significant ($p < 0.05$, χ^2 test); Likert scale: 1 – little or no acquisition of skills; 2 – insufficient acquisition of skills; 3 – acceptable acquisition of skills; 4 – satisfactory level of skill acquisition; H1 – Professional Skills; H2 – Communication and Personal Skills; H3 – Basic Knowledge, Information Management and Critical Thinking Skills; H4 – Ability to Collect Clinical Information; H5 – Ability to Diagnose and Treat; H6 – Ability to Establish and Maintain Health; H7 – Health Promotion Skills.

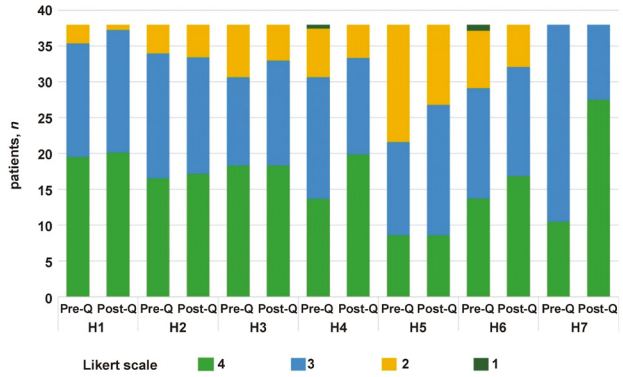


Fig. 1. Comparison of students' perceived competency acquisition before (pre-Q) and after (post-Q) the coronavirus disease 2019 (COVID-19) lockdown

Likert scale: 1 – little or no acquisition of skills; 2 – insufficient acquisition of skills; 3 – acceptable acquisition of skills; 4 – satisfactory level of skill acquisition; H1 – Professional Skills; H2 – Communication and Personal Skills; H3 – Basic Knowledge, Information Management and Critical Thinking Skills; H4 – Ability to Collect Clinical Information; H5 – Ability to Diagnose and Treat; H6 – Ability to Establish and Maintain Health; H7 – Health Promotion Skills.

Item-level analysis within each competency domain

Professional Skills

Table 3 shows the p-values obtained from the χ^2 test for each questionnaire item within this competency domain. The global assessments obtained before and after the lockdown for questions 3 and 8 are identical. Significant changes were detected in the distribution of opinions for questions 1, 2, 4, 5, 6, and 7. Question 5 showed the greatest change, with 21 students assigning the highest score (4) in the pre-Q compared with only 6 students in the post-Q. The mean competency score was 3.44 ±0.62 in the initial questionnaire and 3.51 ±0.54 in the final questionnaire (p = 0.341).

Communication and Personal Skills

Statistically significant differences were observed for questions 10, 13 and 14. For questions 10 and 15, six and five students, respectively, reported lower scores in the post-Q than in the pre-Q. Thirty-three students assigned a score of 3 to question 12 at the end of the course (Table 3). The mean competency score remained unchanged, at 3.33 ±0.65 in the initial questionnaire and 3.33 ±0.68 in the final questionnaire.

Basic Knowledge, Information Management and Critical Thinking Skills

Statistically significant differences were observed for question 18 (Table 3). At the end of the course, 5 students assigned a score of 2 to question 16, whereas more than half of the students assigned the highest score (4) to question 18. The mean competency score increased slightly from 3.30 ±0.77 to 3.35 ±0.70, although this difference was not statistically significant (p = 0.696).

Ability to Collect Clinical Information

With the exception of question 25, significant differences between the pre-Q and post-Q were observed for all questions (Table 3). Question 25 showed an identical distribution of responses at both assessment time points. Question 19 received predominantly the highest rating (4) at the end of the course. In contrast, students' ratings for question 26 declined compared with the baseline assessments. For item 27, although 5 students assigned the lowest possible score in the pre-Q, no student selected this rating in the post-Q. The changes in the perceived competency acquisition across competency domains H1–H4 before and after the lockdown are displayed in Fig. 2. The mean competency score increased from 3.15 ±0.75

Table 3. Differences in responses to individual questionnaire items across competency domains H1–H4 before and after the coronavirus disease 2019 (COVID-19) lockdown

Domain	Item	Pre-Q *Me*	Post-Q *Me*	*p*-value
H1	1	4	4	0.046*
	2	3	4	0.000*
	3	4	4	1.000
	4	3	3	0.000*
	5	4	3	0.000*
	6	4	4	0.014*
	7	3	3	0.046*
	8	4	4	1.000
H2	9	3	4	0.405
	10	3	3	0.014*
	11	3	3	0.180
	12	3	3	0.225
	13	3	3	0.002*
	14	4	4	0.025*
	15	3	4	0.134
H3	16	4	4	1.000
	17	3	3	0.102
	18	3	3	0.001*
H4	19	3	4	0.000*
	20	4	4	0.025*
	21	3	4	0.000*
	22	3	4	0.001*
	23	3	4	0.000*
	24	3	3	0.014*
	25	3	3	1.000
	26	3	3	0.014*
	27	2	3	0.000*

* statistically significant (p < 0.05, Wilcoxon test).

to 3.40 ±0.69, representing a statistically significant improvement (p = 0.008).

Ability to Diagnose and Treat

No statistically significant differences were detected for 2 questionnaire items (Table 4). For question 28, the students reported greater confidence at the end of the course. For question 29, the majority of the students assigned a score of 3 in the post-Q, whereas a score of 2 predominated in the pre-Q. The obtained responses improved significantly between the 2 time points. The mean scores for this ability increased from 2.78 ±0.78 to 2.93 ±0.72, reaching statistical significance (p = 0.048).

Ability to Establish and Maintain Health

Statistically significant differences were detected for most questionnaire items (Table 4). For skills with a p-value of 1.000, the distribution of responses was identical at both time points. However, the evaluations were more positive at the end of the course as a rule. The mean competency score increased from 3.10 ±0.81 to 3.29 ±0.72 ($p < 0.05$).

Health Promotion Skills

Significant differences were observed for both questionnaire items, with considerable improvement in students' perceived competency acquisition between the pre-Q and post-Q. Figure 3 illustrates the changes in perceived competency acquisition for sections H5–H7 before and after the lockdown. The mean score increased from 3.28 ±0.45 to 3.72 ±0.40 ($p < 0.05$).

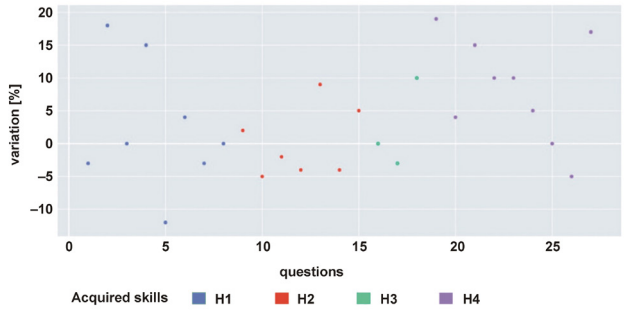


Fig. 2. Variation in students' perceived acquisition of competencies across domains H1–H4 before and after the COVID-19 lockdown

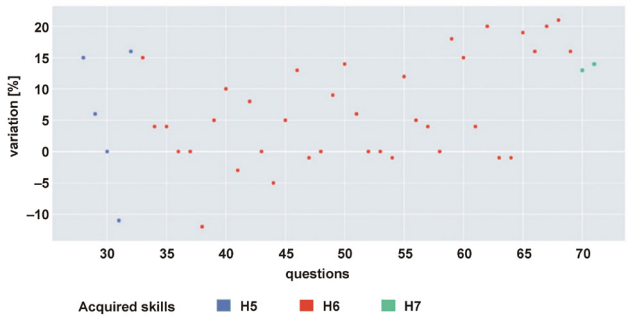


Fig. 3. Variation in students' perceived acquisition of competencies across domains H5–H7 before and after the COVID-19 lockdown

Table 4. Differences in responses to individual questionnaire items across competency domains H5–H7 before and after the coronavirus disease 2019 (COVID-19) lockdown

Domain	Item	Pre-Q *Me*	Post-Q *Me*	*p*-value
H5	28	3	3	0.000*
	29	2	3	0.134
	30	3	3	1.000
	31	3	3	0.001*
	32	2	3	0.000*
H6	33	3	3	0.002*
	34	4	4	0.025*
	35	4	4	0.025*
	36	4	4	1.000
	37	4	4	1.000
	38	4	3	0.000*
	39	3	3	0.014*
	40	3	3	0.001*
	41	3	3	0.317
	42	4	4	0.001*
	43	4	4	1.000
	44	3	3	0.014*
	45	3	4	0.014*
	46	3	4	0.000*
	47	3	3	0.763
	48	3	3	1.000
	49	3	3	0.002*
	50	3	3	0.000*
	51	2	3	0.014*
	52	3	3	1.000
	53	3	3	0.000*
	54	3	3	0.763
	55	2	3	0.001*
	56	3	4	0.014*
	57	4	4	0.025*
	58	3	3	1.000
	59	3	4	0.000*
	60	3	4	0.000*
	61	3	3	0.025*
	62	3	3	0.000*
	63	3	3	0.763
	64	3	3	0.763
	65	3	3	0.000*
	66	3	3	0.000*
	67	2	3	0.000*
	68	2	3	0.000*
	69	2	2	0.025*
H7	70	3	4	0.000*
	71	3	4	0.000*

* statistically significant ($p < 0.05$, Wilcoxon test).

To illustrate in a didactic way the changes in students' perceived competency acquisition before and after the COVID-19 lockdown, the percentage of students reporting changes in their perception was calculated. Figures 2 and 3 present these changes for sections H1–H4 and H5–H7, respectively. Positive percentages indicate an improvement in the acquisition of skills whereas negative percentages indicate a decline in the perceived competency acquisition relative to students' expectations. Each point in Fig. 2 and Fig. 3 represents the variation in perception for an individual questionnaire item and is color-coded accordingly.

Discussion

After analyzing the results, it was concluded that the education received by the students for the proposed competencies was acceptable but insufficient for the development of their future professional career. It is important to bear in mind the period during which the questionnaires were administered, from December 2019 to March 2020. During this time, the students had completed the first academic semester and had started the second semester. The skills in which a greater number of students showed deficiencies and, therefore, required more reinforcement were the Ability to Collect Clinical Information and the Ability to Establish and Maintain Health. When the students assessed their professionalism, it was observed that they felt quite capable in 4 out of the 8 competencies evaluated, with the highest confidence reported for respecting patients and colleagues regardless of gender, origin, socioeconomic status, language, or culture. However, they reported limited confidence in recognizing inappropriate conduct, taking appropriate action and support patients, when necessary. When assessing Communication and Personal Skills, their preparation was acceptable in 3 of the 7 competencies evaluated. They felt highly capable of establishing an adequate dentist–patient relationship required for effective dental care. Their preparation for working with other members of the professional team was also considered good. However, they reported little ability to share verbal or written information with patients and other professionals, including discussion skills, as well as to give and receive constructive criticism. When assessing their Basic Knowledge, Information Management and Critical Thinking Skills, the deficiencies were mainly related to the ability to evaluate scientific research and clinical studies and integrate this information to improve patient's oral health. Regarding the Ability to Collect Clinical Information, the students felt capable only of identifying the patient's chief complaint. However, their preparation for performing diagnostic procedures in oral pathology was, in some cases, perceived as inadequate. None of the students felt sufficiently prepared to diagnose and plan treatments, especially for patients with special needs. The highest-rated competency was the preparation of the clinical history.

Based on our results, it is evident that the collection of clinical information through an interview could be implemented online, but the information obtained through palpation of extraoral structures requires in-person contact, as do the different radiographic techniques required to establish an adequate diagnosis. There is no doubt that the interpretation of radiographic tests does not necessarily require the presence of the patient, and therefore all these activities could be implemented without the presence of the patient in the dental clinic. The students considered that they were quite capable of informing patients about relevant aspects of the pathologies they presented. The largest number of skills that can be acquired in the practicum is based on the establishment and maintenance of health. Of the 37 competencies that must be acquired, the students perceived that they had been adequately trained in 14 of them. These included preventive, cariogenic and periodontal aspects. Their main deficiencies were associated with pharmacological, surgical, prosthetic, orthodontic, and orthopedic therapies. The poor ability to diagnose temporomandibular disorders was noteworthy. The fact that none of the students felt sufficiently prepared to perform extractions of erupted teeth was surprising. Regarding the competencies based on health promotion, the future dentists considered themselves quite or adequately prepared (>90%). Individualized health education and counseling to improve and maintain patients' oral health can be provided through the implemented technologies. In all cases where significant differences were detected, the improvement in the students' assessments was positive, with no significant changes noted for communication and knowledge skills.

In Spain, Congosto et al.[16] conducted a similar study to the present one. However, these authors evaluated the acquisition of student competencies in a non-pandemic setting, where clinical activities were not affected. They determined the students' perception regarding the acquisition of skills at the end of their dental studies, classifying them into 5 groups: professional values; ethical attitudes and behaviors; scientific foundations; acquisition and critical assessment of information; clinical skills such as diagnosis, prognosis, treatment planning and dental therapy; and public health and healthcare systems. In this study, the students felt very competent in identifying patient concerns and expectations, and in communicating effectively and clearly, both verbally and in writing, with patients, relatives and other healthcare professionals. In our research, 45.11% of the students reported being highly trained in communication skills at the end of the academic year, which was similar to the percentage of students who felt qualified in this skill at the beginning of the study. In addition, according to these authors, the students felt very capable of sharing information with other professionals and working as a team. They obtained favorable scores for recognizing ergonomics principles, maintaining workplace safety, and planning and applying appropriate treatments

according to the patient's dental condition. In our case, a high percentage of the students felt that these skills had been acquired at an acceptable level. Congosto et al. detected competency deficits regarding knowledge of the scientific method and critical thinking[16]; however, most of our students reported an acceptable level of these skills.

The pandemic prevented students from attending the university clinic from the beginning of March, practically halving the face-to-face clinical training period. Hence, the professors focused on promoting, through online activities, skills that were not directly related to the development of specific manual abilities of the profession. Since it was impossible to simulate a clinical environment at the students' homes or to perform activities requiring manual skills, professors had to adapt their teaching methods.

The complex organization of the course divided the students into groups of 20–25, facilitating virtual activities and personalized attention.

Different learning methodologies were applied to compensate for the lack of direct patient care, including classroom debates and online clinical situations. Reflection is a key component of professional skills, yet aspects of the healthcare professionals' emotional sphere are left aside.[15]

In the Communication and Personal Skills domain, the students felt able to identify psychological and social components, share information with patients and other professionals, apply stress management principles with patients, and work as a team. It should be noted that these skills are developed throughout the 5-year degree program, so students may feel more confident in the competencies developed before the final year of the program. To acquire these skills, clinical cases were designed to allow students to develop an appropriate treatment plan and subsequently present it during virtual discussions. This activity was complemented with problem-based learning in larger groups, allowing students to discuss therapeutic options within a theoretical framework. As a future option, simulated interviews were proposed so that the student could assume the patient's role to enrich the dentist–patient relationship and identify the patient's needs and objectives. This tool would favor the integration of theoretical knowledge with clinical practice.[17]

Regarding Basic Knowledge, Information Management and Critical Thinking Skills, most students felt acceptably competent in assessing the validity of statements related to the advantages attributed to different instruments, products and techniques, as well as in evaluating scientific studies. A total of 12.5% felt insufficiently prepared to use current electronic communication and information technologies, a problem that, in a situation such as the COVID-19 pandemic, limits the possibilities for planning certain activities. It is essential to know how to use online resources and understand how to evaluate clinical studies and research related to health sciences, since professionals need to remain constantly updated. Even though this competency is developed transversally throughout the degree, it was one of the most complex for students to achieve. Small groups of students were assigned to perform bibliographic searches in scientific journals with a high impact factor and then to synthesize and discuss the collected information virtually.

The competency related to collecting clinical information has an important practical component, being essential for identifying the patient's chief complaint, obtaining the history of the present illness, writing reports and referrals, making additional records, and informing the patient. Almost half of the students considered themselves unable to diagnose oral pathology, including performing biopsy. These deficiencies could not be corrected since the practical aspects of this competency were not addressed; therefore, they should be included in future training. Problem-based learning could be an effective methodology for facilitating the development of these skills in dentistry.[18] The objective structured clinical examination (OSCE) as an educational tool could also help overcome these skill deficiencies thanks to the active involvement of the student in virtual environments.[19]

Regarding the Ability to Diagnose and Treat, at the beginning of the pandemic the students had insufficient and/or acceptable knowledge. Diagnosing and planning treatment priorities for patients with special needs was a particular concern. To address this aspect, the educational system focused on the resolution of virtual clinical cases proposed by the professor and student working groups. Through the virtual campus, the seminars planned at the beginning of the academic year were maintained. To improve this competency, the implementation of clinical simulations involving different common scenarios in dentistry was assessed, as this has been reported to be a very favorable and effective educational tool according to some authors.[16] Infographics and applications such as Kahoot!, Edpuzzle and Socrative were also implemented as highly engaging educational resources. The integration of mobile technology into the virtual classroom is an innovative teaching resource that could also enhance student communication and participation by generating scenarios that support the learning process.[20] A reflective diary could contribute to the acquisition of diagnostic and treatment skills.[21]

Furthermore, within the Ability to Establish and Maintain Health domain, many deficiencies were identified, including the diagnosis of temporomandibular disorders, treatment planning, the design and application of adhesive restorations, crowns, bridges, and removable prostheses, as well as the design, placement and adjustment of space maintainers and active appliances for less complex treatments. In addition, the students reported a lack of confidence in managing orthodontic emergencies, performing simple surgical extractions of unerupted teeth and fractured or retained roots, or referring the patient when necessary. To address this problem, the initial plan was to develop videos with patients or phantoms, but this approach was replaced by practical

online seminars, videoconferences and clinical case discussions through the educational platform. Methods based on solving problems associated with clinical images, such as the WebCT virtual platform,[22] could have contributed to the acquisition of these skills. As a long-term strategy, the implementation of virtual reality could be considered, as it is an appropriate tool for acquiring clinical treatment skills while reducing risks and providing a safe environment for future professionals.[23]

The students did not feel highly competent in developing and implementing health programs and coordinating interinstitutional and interprofessional collaborations. This deficiency was addressed through collaborative work focused on developing individualized protocols based on scientific evidence for different population groups, such as adolescents, adults, older adults, or patients with special needs.

Due to the COVID-19 pandemic, it was necessary to modify the teaching and learning methodology used in clinical practice. Despite students' positive assessment of online resources, partly because of the flexibility they offer and the accessibility of information from anywhere,[24] the acquisition of some competencies proved complex. Information and communication technologies helped students acquire theoretical knowledge, but they contributed only marginally to the acquisition of practical skills. Therefore, new tools should be devised, such as the use of simulators to improve manual skills in exceptional situations such as the COVID-19 pandemic.[25]

However, one of the greatest technological limitations has been the information and communication skills of the faculty staff, which interfered with the achievement of some planned activities. Ethical and technical aspects related to the use of these tools continue to be challenging for professors and students.[26] This experience should be used to provide more evidence regarding positive and unfavorable aspects that technology offers in education and to study the safety of the technological environment in universities. In a systematic review carried out by Naciri et al., the authors observed a lack of consensus among students regarding their perception, motivation and commitment to this type of education.[27]

Another important obstacle was the loss of direct contact with the students, who reported a great overload of work and information received by e-mail. The literature includes studies that analyze the impact of the COVID-19 pandemic on health, both orofacial[28] and psychological.[29–31] This loss of contact, possibly exacerbated by a lack of motivation and acceptance, could be responsible for the loss of students who were willing to participate in the second questionnaire, thus affecting the total sample size of the study. This inconvenience is consistent with the results reported by other authors, such as Kivlehan et al.[32] and Singhi et al.[33] who, despite initially recruiting larger samples, reported final sample sizes of only 30 subjects due to participant loss during data collection.

One aspect that was not taken into account, but which some authors have highlighted, was the influence of professors' beliefs and knowledge on teaching strategies.[34]

This exceptional situation has led us to rethink our methodology for future situations in which face-to-face teaching or clinical practice with patients cannot be provided.

Mentoring could have been an additional strategy to improve the acquisition of skills and attitudes by giving students greater self-confidence, self-knowledge and the ability to establish priorities.[35]

One of the most important limitations of the present study is the small sample size. Considering that completion of the questionnaires was voluntary and that participation did not entail any economic or academic reward, the lack of participation of all enrolled students is understandable. To ensure the transparency of the reported results, a post hoc analysis for paired samples, taking into account the statistical test used, was carried out using the G*power software. The result indicated a small effect size (0.24) and statistical power of 30%. This has motivated the researchers to start new projects with larger sample sizes and methodologies designed to encourage student participation.

This research is pioneering in its field because the pandemic provided a unique opportunity to evaluate the students' perception of their ability to acquire competencies necessary for future professional practice without traditional face-to-face clinical practice or advanced face-to-face simulation activities. We believe that the excellent results obtained under these circumstances could be due to the fact that these same students had previously completed fourth-year courses involving clinical practice with patients.

Based on the present research, it can be affirmed that patient management cannot be replaced entirely by online teaching, but online teaching can complement traditional teaching. Medical educators and students have also stated that they are in favor of the face-to-face modality and perceive online learning as appropriate for theoretical teaching and some clinically oriented activities, especially as a vehicle for the discussion of clinical cases.[36] However, according to research in the field of health professions education, there is still no consensus, and researchers agree on the need for further research to overcome the current lack of studies that comprehensively address these issues during pandemic.[26]

It must be emphasized that the last phase of this study was not planned at the beginning of the investigation and was initiated in response to the pandemic.

Conclusions

Based on the perception of fifth-year students enrolled in the Dentistry degree, despite the challenges posed by the pandemic, they consider that by the end of the academic year they had acquired specific competencies related to

professional skills, collection of clinical information, diagnosis, treatment, the establishment and maintenance of health, and health promotion. A general improvement in the students' perception of competency acquisition between the 2 assessment time points was observed.

Future actions should focus on improving communication skills, personal skills, basic knowledge, information management, and critical thinking.

The global pandemic forced the methodologies of the Integrated Dental Clinic course to be modified, shifting from a fully practical and patient-oriented approach to a new online version, in which the student was the center of the learning process.

Taking into account the improvement observed in certain skills during the course of the program, it is obvious that, with the online technological resources implemented during the pandemic, learning is insufficient to provide patients with optimal dental care. However, we are prepared to replace some learning activities that have traditionally been performed in the presence of the patient, thereby contributing to environmental sustainability.

Ethics approval and consent to participate

The present study was reviewed and approved by the Ethics Committee of the Quality Office of the Complutense University of Madrid, Spain (code No. 375-2019). The study was conducted in accordance with the principles of the Declaration of Helsinki, the recommendations of the World Association of Medical Editors (WAME), the International Committee of Medical Journal Editors (ICMJE) guidelines for the reporting and elaboration of research, and the Spanish Organic Law 3/2018 of December 5 on the Protection of Personal Data and Guarantee of Digital Rights (LOPD).

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

State of oral health and the impact of regular physical activity on selected salivary parameters in young athletes in relation to the sport-specific environment

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Abstract

Background. Poor oral health is correlated with participation in various sports and has a significant impact on athletes' quality of life (QoL).

Objectives. The aim of the present study was to evaluate oral health and selected salivary parameters in young athletes in relation to the environment of the performed sport.

Material and methods. Sixty adolescents aged 12–15 years (mean age: 13.0 ±0.5 years), including 30 competitive swimmers and 30 competitive basketball players, participated in the study. The following indices were calculated: the number of teeth affected by caries – decayed, missing and filled teeth (DMFT); and the simplified oral hygiene index (OHI-S). Saliva samples were collected from all subjects before and after the training. The saliva flow rate (V), pH, buffer capacity, and calcium (Ca) level, as well as salivary alpha-amylase (sAA) and salivary peroxidase (sPO) concentrations, were measured. In addition, the athletes rated the intensity of the training session using Foster's rating of perceived exertion (RPE) 0–10 scale.

Results. The mean DMFT score was 4.53 ±3.54 in the swimmers and 3.43 ±2.45 in the basketball players. The mean OHI-S score in the swimmers was 0.73 ±0.58, which was significantly higher than in the case of the basketball players (0.34 ±0.37). Some fluctuations in the salivary parameters were noticed in the swimmers vs. the basketball players, which was related to the type of training. When comparing the swimmers with the basketball players after the training, a significantly lower Ca level was observed in the latter group. However, when comparing post-training changes in the salivary parameters with regard to the pre-training values, significant decreases in V and increases in pH were observed in both groups. After the training, significant increases in the Ca level and the sAA concentration were found in the swimmers. The average RPE score was 4.96 ±2.16 in the swimmers vs. 5.13 ±2.02 in the basketball players. Most swimmers perceived the training as 'somewhat hard' or 'hard', and so did the basketball players.

Conclusions. While the data showed no significant differences in DMFT between the groups, the basketball players presented significantly better oral hygiene in comparison with the swimmers. The data suggests that the salivary V and pH, and the levels of Ca and sAA are associated with the swimming training session, whereas V and pH are also associated with the basketball training session. The regression model showed that the sAA concentration could be a predictor of OHI-S, and that the salivary V, pH, buffer capacity, and Ca level could be predictors of the effort rating among swimmers. Also, pH could be a predictor of DMFT in basketball players.

Keywords: saliva, youth athletes, rating of perceived exertion, DMFT, OHI-S

Highlights

- No significant differences were observed in the decayed, missing and filled teeth index (DMFT) values between the 2 study groups; however, basketball players demonstrated better oral hygiene.
- In both athlete groups, a decrease in the salivary flow rate (V) and an increase in the salivary pH were observed following the training.
- In swimmers, the salivary alpha-amylase (sAA) levels were positively correlated with the simplified oral hygiene index (OHI-S) values, whereas physical exertion showed a negative correlation with the salivary V and calcium (Ca) levels.
- The salivary V, pH, and Ca and sAA levels were associated with the swimming training.
- Both athlete groups rated the training as 'moderately difficult' or 'difficult'.

Introduction

Oral health is now recognized as one of the main factors directly influencing overall health, which subsequently determines the person's quality of life (QoL) through well-being and physical fitness.[1]

The oral cavity is considered the primary site of inflammatory outbreaks. Studies have shown that 80% of the seeding sources of pathogenic microorganisms are located in the head area, and of these, more than 90% involve the oral cavity.[2] Oral pathologies, such as dental caries disease and periodontal disease, among others, are correlated with the practice of various sports and have a significant impact on athletes' QoL. Athletes should, therefore, pay more attention to their oral health in order to improve performance.[3] According to the World Health Organization (WHO), dental caries is defined as a pathological process of localized external origin, leading to the decalcification of enamel and dentin, the breakdown of the hard tissues of the tooth, and subsequent cavity formation.[4] It is considered one of the most common global health problems.[5] It is assumed that dental caries is due to the imbalance between the alternating demineralization and remineralization processes at the enamel–dental plaque–saliva interface.

Caries develops as a result of the accumulation of plaque on the tooth surface. The bacteria in the plaque metabolize the carbohydrates supplied by the diet into acids, leading to the lowering of pH in the mouth and the plaque, and consequently to the demineralization of the tooth tissues. Bacteria that cause tooth decay are mainly *Streptococcus mutans*, *Streptococcus sobrinus* and lactic acid bacilli. Individuals with poor oral hygiene show higher rates of caries.[6]

Athletes themselves often have a dismissive attitude toward oral health.[7] Obsessive calorie counting to maintain weight as a goal to achieve better and better performance in sports leads to eating disorders that are often hidden in the sports community and are difficult to diagnose.[8]

One of the main factors playing a very important role in keeping the oral microflora in balance is saliva.[9] The constant flow of saliva removes food debris and microorganisms from the oral cavity. The buffering effect (acid neutralization) maintains the proper acid–base balance through the regulation of pH. Depending on oral hygiene habits, dietary habits and the buffering action of saliva, the pH values vary from 5.7 to 6.2 at rest and 8.0 as a result of stimulation.[3] When pH falls below the critical value of 5.5, the superficial demineralization of the tooth tissues occurs. The remineralization capacity of the hard dental tissues is linked with the content of calcium (Ca), phosphate and fluoride ions in saliva, among other things.[10,11] At pH = 5.5, saliva is a saturated solution of these ions. Apart from the aforementioned salivary parameters, also salivary alpha-amylase (sAA) and salivary peroxidase (sPO) have a protective effect on oral health.

Salivary alpha-amylase is the predominant enzyme found in saliva, and also in plaque. It is one of the proteins that can bind to several oral bacterial species, thus inhibiting their adhesion to various surfaces in the mouth. The enzyme is responsible for breaking down the ingested starch and glycogen into maltose.[9,12] Furthermore, sAA is used as a psychophysiological stress marker during endurance exercise in various sports.[13] The sAA levels increase in response to stress-related activities. This reaction is triggered by the elevated levels of catecholamines (norepinephrine and adrenaline), which are released into the bloodstream under stress conditions.[14]

Salivary peroxidase, on the other hand, has a beneficial effect on the oral microflora. It participates in the antimicrobial protection of all surfaces in the oral cavity. It has a bacteriostatic, and even bactericidal effect on carious bacteria.[12,15]

Numerous studies have documented that exercise significantly affects the saliva secretion and composition.[12,16–18] The reduction in the saliva secretion during intensive exercise is explained by an increase in the sympathetic innervation activity, which causes the constriction of the vessels supplying blood to the salivary glands, as well as an increase in the level of vasopressin (an antidiuretic hormone) and, consequently, a lower salivary volume.[19,20] What cannot be overlooked is the effect

of dehiscence, defined as the disruption of the continuity of the oral mucosal tissue, and mouth breathing during exercise. These factors can lead to saliva evaporation and hyperventilation.[20,21]

Basketball players are known to experience greater dehydration of the body as compared to swimmers, which also affects the volume and composition of saliva, and may translate into oral health and the occurrence of dental caries in particular.

Saliva analysis has been shown to have some medical and practical advantages. From a medical point of view, saliva collection is a non-invasive method with a reduced risk of possible infections as compared to other methods, such as blood analysis.[22,23] From a practical point of view, in comparison with invasive methods, a generally lower cost and athletes' tolerance are among the advantages of using saliva markers, which may be particularly applicable in the assessment of a cohort of young athletes.[22]

Saliva used as a diagnostic tool, and particularly determining its secretion rate, buffer capacity, pH value, and the growth of carious bacteria, is applied in caries risk assessment.[3,5,24] Saliva can also serve as an alternative diagnostic material to monitor the response of the body to the physical exertion associated with sports activities.[25]

Monitoring the body's response to different types of exercise is significant in modifying the performance of exercisers while preventing undesirable situations, such as fatigue and overtraining.[26] Monitoring the training load during daily sessions with the use of common physiological measurements, such as the heart rate, the blood lactate concentration or the oxygen uptake, is challenging. Alternatively, non-invasive biochemical measures to assess exercise intensity may include salivary cortisol, total protein and sAA.[3,27]

However, the simplest form of assessing exercise intensity is the reporting of the perceived exertion. The exerciser expresses their subjective, post-training sensations of discomfort and fatigue.[28] Several methods have been developed for the subjective assessment of effort. Foster et al. proposed a modified Borg scale based on a 10-point rating of perceived exertion (RPE), which comprises both the intensity and duration of the training, allowing the determination of the training load of the so-called session-RPE.[29] This scale has been used in the assessment of young athletes' training.[18,30,31]

A number of studies on the monitoring of salivary biomarkers in the athletes of team sports, such as basketball, volleyball and soccer, and individual sports, such as running and swimming in a specific aquatic environment, are available.[17,18,32–36] However, an area that still requires more investigation are the younger age categories. In the context of the popularity of sports such as swimming and basketball among young athletes, it is important to understand what load is placed on the athletes' bodies during individual training sessions. The swimming training is characterized by the endurance nature of the effort undertaken in a specific aquatic environment and based on the predominant aerobic metabolism.[37,38] Basketball, on the other hand, is a contact sport characterized by variable intensity and high dynamics of the undertaken technical and tactical actions, with performance depending on physical aptitude (i.e., power, speed, agility, and endurance).[39] It is a team sport, and thus psychological (mood, motivation) and social factors (relationships, team microclimate) influence the level of performance.[40] Differences in the specific conditioning and physical demands of swimming and basketball suggest that the training sessions may induce different internal stresses in exercisers, which can be understood as subjective responses and objective changes in the salivary biomarker concentrations.

A number of studies have found that playing competitive sports reduces the risk of cardiovascular disease and diabetes, helps control body weight, enhances physical fitness and joint mobility, and improves sleep quality.[41] According to the few published papers, poor oral health can negatively affect overall athletic performance and the training itself.[42–45] However, according to the World Dental Federation (FDI) guidelines, a healthy mouth is an important factor in helping athletes perform at their best.[46]

Despite a growing body of research on the effect of exercise on biochemical changes in saliva, little information is available on the oral health of adolescents in competitive sports.

The aim of the present study was to evaluate the dental and oral hygiene status, and the effect of physical activity on selected salivary parameters, depending on the environment of the sports practiced.

Material and methods

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

The study included 60 adolescents aged 12–15 years (mean age: 13.0 ±0.5 years), half of whom (n = 30) were competitive swimmers (23 males and 7 females) and the other half (n = 30) trained in basketball (30 males). This study was a quasi-experiment. The inclusion criteria comprised swimmers and basketball players recruited from 2 sports championshipclubs in swimming and basketball operating in the city. All subjects had to provide their parents' or legal guardians' written consent to be able to participate in the study. They had to be willing to submit to the collection of a saliva sample and answer the questionnaire. Those who did not meet the inclusion criteria were excluded from the study.

Both the swimming and basketball training was held 5 days a week, Monday through Friday. The subjects had

trained for 2–5 years. The test was conducted at the same time of day in both study groups. The test was conducted on Tuesday. It referred to one training session. Each athlete was tested once. The study was based on a questionnaire containing questions about the diet, eating and circadian habits, the RPE questionnaire regarding the subjective evaluation of the perceived exertion after one training session, and laboratory tests of the saliva collected before and after the training session, conducted by a pediatric dentist with 24 years of professional experience.

Each swimming training session lasted 105 min in the afternoon (from 4:00 to 5:45 p.m.). The swimming workout session consisted of swimming a distance of 5,000 m. Each routine 90-minute basketball training session included a 15-minute warm-up, 60 min of physical, technical and tactical exercises, and 15 min of game play. The training sessions were held at an indoor basketball court in the afternoon (from 4:00 to 5:30 p.m.). Prior to the training, each subject was instructed to refrain from drinking during the training session, and underwent a clinical examination with a standard mirror and a probe under artificial lighting. The examination included the assessment of the condition of the tooth tissues in terms of caries disease, and the evaluation of oral hygiene in terms of plaque and calculus.

The condition of the teeth was assessed according to the WHO criteria based on the number of decayed (DT), missing (MT) and filled teeth (FT); the DMFT index was calculated for each subject and the prevalence of caries in both groups was established. The oral hygiene status was assessed using the simplified oral hygiene index (OHI-S) for plaque/debris (DI-S) and calculus (CI-S) separately. The presence of dental deposits on the buccal surfaces of teeth 16, 11, 26, and 31, and on the lingual surfaces of teeth 36 and 46 was evaluated.

The evaluation of the presence of dental deposits was carried out using a dental probe. The assessment of the presence of plaque and calculus was based on a scale from 0 to 3, where 0 means no plaque and no calculus, 1 – plaque or supragingival calculus covering less than ⅓ of the tooth surface, 2 – plaque or supragingival calculus covering less than ⅔ of the tooth surface, or the presence of single points of subgingival calculus, and 3 – plaque or supragingival calculus covering more than ⅔ of the tooth surface, or a thick band of subgingival calculus around the tooth neck. The values for each indicator (DI-S and CI-S) were obtained separately by summing up the scores for each tooth surface and dividing the total score by the number of examined surfaces. To obtain OHI-S, the DI-S and CI-S values were added.

Salivary sample collection and the studied parameters

All unstimulated mixed saliva samples were collected from the swimmers and basketball players only once in the afternoon, before and after the swimming and basketball training session. All subjects were asked to rinse their mouths with water before saliva collection. The total number of saliva samples was 120. During sampling, the subjects sat with their heads bowed and mouths open. Saliva was collected with a plastic pipette and put into a graduated tube placed on crushed ice. The saliva flow rate (V) was calculated in mL/min, through measuring the volume of the collected sample and the time required for its collection. The samples were centrifuged at 3,500 rpm for 10 min before biochemical tests were performed. The following salivary parameters were evaluated through laboratory tests: pH; buffer capacity in mmol/L (the potentiometric method); the Ca level in mg/L (the method based on the formation of a chromogenic complex between the Ca ions and o-Cresolphthaelin – a commercial kit; Alpha Diagnostics, Warsaw, Poland); sAA in IU/L (Caraway's colorimetric method – commercial kit; Alpha Diagnostics); and sPO in mIU/mL (the Nbs-SCN method).[47]

In addition, training intensity was self-assessed by the swimmers and basketball players using Foster's scale – a modified Borg CR-10 scale, which is a category-ratio RPE scale. It relates the athlete's perception of effort to a numerical score (from 0 – rest to 10 – maximal).[28]

Foster's RPE scale was used about 30 min after the training session to assess the internal load of the entire training session. The training load was dichotomously categorized as light (scores 0–4) or high (scores 5–10).

Statistical analysis

Depending on the type of variable distribution, either Student's t test or the Mann–Whitney U test was used for the analysis of independent variables. For dependent variables, either repeated measures Student's t test or non-parametric repeated measures Wilcoxon's test was conducted.

Spearman's correlation coefficient (ρ) was calculated with regard to the RPE scores and changes in the salivary parameters before and after the training session. Similarly, the correlations between the DMFT scores, as well as the OHI-S scores, and changes in the salivary parameters before and after the training session were examined.

A logistic regression analysis was performed to determine the salivary parameters which had an influence on the dental and oral hygiene status.

Bivariate logistic regression analyses were carried out for the swimmers and the basketball players, for both independent (the salivary parameters) and dependent variables (DMFT and OHI-S, and light or high training load according to Foster's RPE scale).

The level of significance was set at $p < 0.05$. All analyses were performed with the use of the Statistica software package, v. 13.3 (StatSoft, Inc., Tulsa, USA).

Results

DMFT and caries prevalence

The prevalence of caries in the swimmers was 56.7%, while in the basketball group, it was 66.7% (Fig. 1).

In the group of swimmers, the following mean scores were recorded: DT = 1.20 ±1.27; MT = 0.00 ±0.00; and FT = 3.33 ±3.19. On the other hand, in the group of basketball players, the mean values of the DMFT components were as follows: DT = 1.10 ±1.04; MT = 0.00 ±0.00; and FT = 2.33 ±2.34. In total, the mean DMFT score was 4.53 ±3.54 in the swimmers and 3.43 ±2.45 in the basketball players. The mean (*M*), standard deviation (*SD*), median (*Me*), as well as minimum (min) and maximum (max) values for DT, MT, FT, and DMFT are shown in Table 1. There were no statistically significant differences in DMFT and dental caries incidence between the groups.

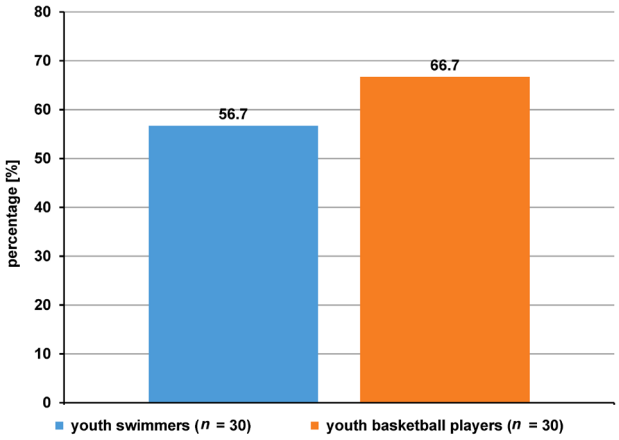


Fig. 1. Dental caries prevalence in the studied groups

OHI-S

For the group of swimmers, the mean OHI-S score was 0.73 ±0.58, which was significantly higher (p = 0.006) as compared to the group of basketball players with a mean OHI-S value of 0.34 ±0.37. Supragingival calculus was present only in the swimmers, while plaque was present in both groups (0.62 ±0.46 vs. 0.39 ±0.41, respectively), with a significantly higher value in the group of swimmers (p = 0.035) (Table 2).

Salivary parameters before and after the training session in swimmers

After the training session, the salivary V showed a significant decrease (p = 0.009), while significant increases in pH (p = 0.013), Ca (p = 0.001) and sAA (p = 0.028) were observed. Moreover, after the training, a decrease in buffer capacity and an increase in sPO were noted; however, the changes were not statistically significant (Table 3).

Salivary parameters before and after the training session in basketball players

After the training session, the salivary V showed a significant decrease (p = 0.047), while there was a significant increase in pH (p = 0.011). Furthermore, after the training session, decreases in buffer capacity, Ca and sPO, as well as an increase in sAA, were observed; however, the changes were not statistically significant (Table 3).

Table 1. Values of the decayed, missing and filled teeth index (DMFT) and its components for the studied groups

Dental status	Youth swimmers (n = 30)			Youth basketball players (n = 30)			*p*-value
	M ±*SD*	*Me*	min–max	*M* ±*SD*	*Me*	min–max	
DT	1.20 ±1.27	1.00	0.00–4.00	1.10 ±1.04	1.00	0.00–4.00	0.935
MT	0.00 ±0.00	0.00	0.00–0.00	0.00 ±0.00	0.00	0.00–0.00	–
FT	3.33 ±3.19	3.00	0.00–12.00	2.33 ±2.34	2.00	0.00–10.00	0.224
DMFT	4.53 ±3.54	4.00	0.00–15.00	3.43 ±2.45	3.00	0.00–10.00	0.159

M – mean; *SD* – standard deviation; *Me* – median; min – minimum; max – maximum; DT – decayed teeth; MT – missing teeth; FT – filled teeth (Mann–Whitney *U* test). DMFT = (DT + MT + FT) / number of examined people

Table 2. Values of the simplified oral hygiene index (OHI-S) and its components for the studied groups

Oral hygiene status	Youth swimmers (n = 30)			Youth basketball players (n = 30)			*p*-value
	M ±*SD*	*Me*	min–max	*M* ±*SD*	*Me*	min–max	
DI-S	0.62 ±0.46	0.66	0.00–2.00	0.39 ±0.41	0.33	0.00–1.33	0.035*
CI-S	0.11 ±0.22	0.00	0.00–1.00	0.00 ±0.00	0.00	0.00–0.00	0.014*
OHI-S	0.73 ±0.58	0.66	0.00–2.16	0.34 ±0.37	0.24	0.00–1.33	0.006*

DI-S – simplified debris index; CI-S – simplified calculus index; * statistically significant (Mann–Whitney *U* test).

Table 3. Concentration of the test components in the saliva samples collected from the study participants

Salivary parameters	Swimmers vs. basketball players		Swimmers before vs. after the training session	Basketball players before vs. after the training session
	before	after		
V [mL/min]	0.63 ±0.63 0.49 ±0.23 $u = 0.450$ $p = 0.654^{b}$ (↓22%)	0.49 ±0.39 0.36 ±0.16 $u = 0.754$ $p = 0.449^{b}$ (↓26%)	$z = 2.606$ $p = 0.009^{c*}$ (↓22%) –	$z = 1.984$ $p = 0.047^{c*}$ (↓26%) –
pH	7.13 ±0.33 7.31 ±0.35 $t = -1.997$ $p = 0.050^{a*}$ (↑2%)	7.27 ±0.24 7.51 ±0.26 $t = -3.732$ $p = 0.000^{a*}$ (↑3%)	$t = -2.647$ $p = 0.013^{a*}$ (↑2%) –	$t = -2.712$ $p = 0.011^{a*}$ (↑3%) –
Buffer capacity [mmol/L]	4.98 ±2.63 5.26 ±2.14 $u = -0.280$ $p = 0.774^{b}$ (↑5%)	4.48 ±1.90 4.63 ±2.47 $u = -0.243$ $p = 0.808^{b}$ (↑3%)	$z = 1.278$ $p = 0.201^{c}$ (↓10%) –	$z = 1.326$ $p = 0.184^{c}$ (↓12%) –
Ca [mg/L]	3.72 ±1.34 4.39 ±2.12 $u = -1.249$ $p = 0.213^{b}$ (↑15%)	4.61 ±1.58 3.92 ±1.67 $u = 1.944$ $p = 0.050^{b*}$ (↓15%)	$t = -4.439$ $p = 0.001^{a*}$ (↑19%) –	$z = 1.203$ $p = 0.228^{c}$ (↓11%) –
sAA [IU/L]	111.53 ±120.13 162.30 ±120.57 $u = -2.032$ $p = 0.041^{b*}$ (↑31%)	130.91 ±161.14 193.83 ±115.00 $u = -3.274$ $p = 0.000^{b*}$ (↑32%)	$z = 2.190$ $p = 0.028^{c*}$ (↑15%) –	$z = 1.244$ $p = 0.213^{c}$ (↑19%) –
sPO [mIU/mL]	1.60 ±1.47 0.85 ±0.92 $u = 3.393$ $p = 0.005^{b*}$ (↓47%)	1.73 ±1.65 0.76 ±0.65 $u = 3.622$ $p = 0.001^{b*}$ (↓56%)	$z = 1.121$ $p = 0.262^{c}$ (↑7.5%) –	$z = 0.133$ $p = 0.893^{c}$ (↓11%) –

Data presented as *M* ±*SD*.
V – salivary flow rate; Ca – calcium; sAA – salivary alpha-amylase; sPO – salivary peroxidase; * statistically significant ([a] Student's *t* test, [b] Mann–Whitney *U* test, [c] Wilcoxon's test).

Salivary parameters in swimmers before the training session as compared to basketball players

Higher salivary V and sPO were found in the swimmers before the training session as compared to the basketball players, with only the difference in sPO being statistically significant ($p = 0.005$). In contrast, lower values of pH, buffer capacity, Ca, and sAA were observed before the training session in the swimmers as compared to the basketball players. Among these results, only the differences in pH and sAA were statistically significant ($p = 0.050$ and $p = 0.041$, respectively) (Table 3).

Salivary parameters in swimmers after the training session as compared to basketball players

Statistically significantly higher values of the salivary parameters were observed in the swimmers after the training session for Ca ($p = 0.050$) and sPO ($p = 0.001$), and lower for pH ($p = 0.000$) and sAA ($p = 0.000$) as compared to the basketball players (Table 3).

Assessment of the perceived exertion

The mean value on Foster's RPE scale for youth swimmers was 4.96 ±1.75, and the scores ranged from 0 to 10. Most youth swimmers perceived the training as 'moderate' (score 3; $n = 5$), 'somewhat hard' (score 4; $n = 5$) or 'hard' (score 5–6; $n = 15$). The mean value on Foster's RPE scale for youth basketball players was 5.13 ±1.91, and the values ranged from 0 to 10, too. Most youth basketball players perceived the training as 'somewhat hard' (score 4; $n = 10$) or 'hard' (score 5–6; $n = 10$). The distribution of the RPE scores among the participants, and the percentages of athletes according to the perceived light and heavy training load are presented in Fig. 2 and Fig. 3, respectively.

Correlation between changes in the salivary parameters in swimmers and basketball players before and after the workout session

The analysis of correlation between the pre- and post-training salivary parameters showed a significant positive relationship between V and pH ($\rho = 0.550$; $p = 0.002$), and between buffer capacity and sAA ($\rho = 0.416$; $p = 0.022$) in the swimmers. In the basketball players, a significant positive correlation was noticed between buffer capacity and Ca ($\rho = 0.364$; $p = 0.048$), and between sPO and pH ($\rho = 0.457$; $p = 0.011$). Moreover, a significant negative correlation was found in the group of basketball players between sAA and sPO ($\rho = -0.362$; $p = 0.050$), as well as between sPO and V ($\rho = -0.363$; $p = 0.048$) (Table 4).

Correlation between changes in the salivary parameters and the DMFT and OHI-S scores in swimmers and basketball players before and after the workout

No correlation was found between the salivary parameters and the indicators of dental caries in swimmers. However, the analysis of Spearman's correlation between the pre- and post-training salivary parameters and the

Table 4. Spearman's correlation between changes in the salivary parameters in youth swimmers and basketball players before and after the workout session

Group	Salivary parameters	V	pH	Buffer capacity	Ca	sAA	sPO
Youth swimmers	V	–	$\rho = 0.550$ $p = 0.002$*	$\rho = 0.030$ $p = 0.873$	$\rho = -0.084$ $p = 0.658$	$\rho = 0.128$ $p = 0.502$	$\rho = 0.317$ $p = 0.088$
	pH	$\rho = 0.142$ $p = 0.455$	–	$\rho = 0.102$ $p = 0.593$	$\rho = 0.152$ $p = 0.424$	$\rho = -0.056$ $p = 0.769$	$\rho = 0.032$ $p = 0.867$
	buffer capacity	$\rho = -0.098$ $p = 0.605$	$\rho = -0.080$ $p = 0.675$	–	$\rho = -0.028$ $p = 0.885$	$\rho = 0.416$ $p = 0.022$*	$\rho = 0.096$ $p = 0.615$
	Ca	$\rho = -0.289$ $p = 0.121$	$\rho = -0.132$ $p = 0.488$	$\rho = 0.088$ $p = 0.643$	–	$\rho = 0.102$ $p = 0.591$	$\rho = 0.026$ $p = 0.890$
	sAA	$\rho = 0.166$ $p = 0.381$	$\rho = 0.076$ $p = 0.690$	$\rho = -0.010$ $p = 0.959$	$\rho = -0.010$ $p = 0.958$	–	$\rho = 0.059$ $p = 0.758$
	sPO	$\rho = 0.092$ $p = 0.628$	$\rho = 0.302$ $p = 0.104$	$\rho = 0.027$ $p = 0.887$	$\rho = 0.188$ $p = 0.319$	$\rho = 0.168$ $p = 0.374$	–
Youth basketball players	V	–	$\rho = 0.374$ $p = 0.238$	$\rho = 0.202$ $p = 0.284$	$\rho = -0.195$ $p = 0.301$	$\rho = 0.044$ $p = 0.819$	$\rho = -0.312$ $p = 0.093$
	pH	$\rho = -0.054$ $p = 0.775$	–	$\rho = -0.246$ $p = 0.191$	$\rho = -0.069$ $p = 0.715$	$\rho = 0.188$ $p = 0.319$	$\rho = 0.020$ $p = 0.917$
	buffer capacity	$\rho = -0.192$ $p = 0.309$	$\rho = -0.211$ $p = 0.262$	–	$\rho = 0.364$ $p = 0.048$*	$\rho = 0.069$ $p = 0.715$	$\rho = -0.071$ $p = 0.708$
	Ca	$\rho = 0,058$ $p = 0.760$	$\rho = 0.054$ $p = 0.778$	$\rho = -0.138$ $p = 0.469$	–	$\rho = -0.066$ $p = 0.729$	$\rho = 0.139$ $p = 0.464$
	sAA	$\rho = -0.006$ $p = 0.973$	$\rho = -0.201$ $p = 0.287$	$\rho = -0.118$ $p = 0.535$	$\rho = 0.138$ $p = 0.467$	–	$\rho = -0.362$ $p = 0.050$*
	sPO	$\rho = -0.363$ $p = 0.048$*	$\rho = 0.457$ $p = 0.011$*	$\rho = -0.187$ $p = 0.323$	$\rho = -0.320$ $p = 0.085$	$\rho = -0.188$ $p = 0.319$	–

ρ (rho) – Spearman's correlation coefficient; * statistically significant ($p < 0.05$).

Table 5. Spearman's correlation between changes in the salivary parameters and the decayed, missing and filled teeth index (DMFT) and simplified oral hygiene index (OHI-S) scores in youth swimmers and basketball players before and after the workout session

Group	Salivary parameters	DT	MT	FT	DMFT	DI-S	CI-S	OHI-S
Youth swimmers	V	$\rho = 0.105$ $p = 0.579$	–	$\rho = -0.028$ $p = 0.882$	$\rho = 0.039$ $p = 0.839$	$\rho = 0.083$ $p = 0.664$	$\rho = -0.059$ $p = 0.758$	$\rho = 0.063$ $p = 0.734$
	pH	$\rho = -0.149$ $p = 0.433$	–	$\rho = -0.332$ $p = 0.073$	$\rho = -0.294$ $p = 0.114$	$\rho = -0.106$ $p = 0.578$	$\rho = 0.080$ $p = 0.675$	$\rho = 0.090$ $p = 0.635$
	buffer capacity	$\rho = 0.041$ $p = 0.831$	–	$\rho = 0.268$ $p = 0.152$	$\rho = 0.236$ $p = 0.208$	$\rho = -0.037$ $p = 0.844$	$\rho = -0.022$ $p = 0.907$	$\rho = 0.017$ $p = 0.929$
	Ca	$\rho = -0.120$ $p = 0.527$	–	$\rho = 0.091$ $p = 0.631$	$\rho = 0.017$ $p = 0.929$	$\rho = -0.097$ $p = 0.610$	$\rho = -0.093$ $p = 0.624$	$\rho = -0.079$ $p = 0.679$
	sAA	$\rho = 0.151$ $p = 0.427$	–	$\rho = 0.306$ $p = 0.100$	$\rho = 0.196$ $p = 0.298$	$\rho = 0.457$ $p = 0.011$*	$\rho = 0.313$ $p = 0.092$	$\rho = 0.476$ $p = 0.007$*
	sPO	$\rho = 0.191$ $p = 0.313$	–	$\rho = -0.249$ $p = 0.183$	$\rho = -0.106$ $p = 0.578$	$\rho = -0.017$ $p = 0.927$	$\rho = 0.167$ $p = 0.378$	$\rho = -0.039$ $p = 0.838$
Youth basketball players	V	$\rho = 0.242$ $p = 0.198$	–	$\rho = 0.199$ $p = 0.292$	$\rho = 0.219$ $p = 0.245$	$\rho = 0.046$ $p = 0.811$	–	$\rho = 0.072$ $p = 0.705$
	pH	$\rho = 0.069$ $p = 0.716$	–	$\rho = 0.169$ $p = 0.372$	$\rho = 0.201$ $p = 0.285$	$\rho = 0.131$ $p = 0.487$	–	$\rho = -0.027$ $p = 0.887$
	buffer capacity	$\rho = -0.444$ $p = 0.014$*	–	$\rho = -0.184$ $p = 0.330$	$\rho = -0.342$ $p = 0.063$	$\rho = 0.011$ $p = 0.953$	–	$\rho = 0.008$ $p = 0.964$
	Ca	$\rho = 0.014$ $p = 0.942$	–	$\rho = -0.115$ $p = 0.543$	$\rho = -0.108$ $p = 0.571$	$\rho = -0.267$ $p = 0.153$	–	$\rho = -0.190$ $p = 0.315$
	sAA	$\rho = 0.042$ $p = 0.827$	–	$\rho = 0.043$ $p = 0.820$	$\rho = 0.020$ $p = 0.915$	$\rho = 0.054$ $p = 0.777$	–	$\rho = 0.025$ $p = 0.896$
	sPO	$\rho = 0.112$ $p = 0.557$	–	$\rho = 0.074$ $p = 0.698$	$\rho = 0.177$ $p = 0.350$	$\rho = -0.248$ $p = 0.186$	–	$\rho = -0.117$ $p = 0.540$

* statistically significant ($p < 0.05$).

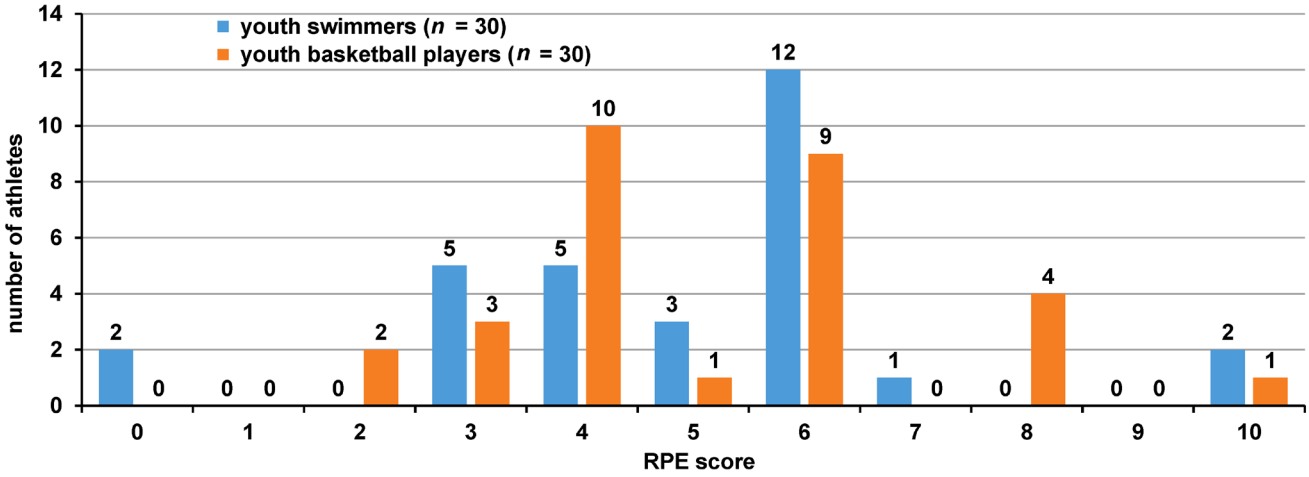


Fig. 2. Distribution of the rating of perceived exertion (RPE) scores after the workout session in the studied groups
Category-ratio RPE scale: 0 – rest; 1–2 – very easy; 3 – moderate; 4 – somehow hard; 5–6 – hard; 7–9 – very hard; 10 – maximal.

oral health status showed a significant positive relationship between sAA and oral hygiene (DI-S and OHI-S) (ρ = 0.457; p = 0.011 and ρ = 0.476; p = 0.007, respectively) in the swimmers. The analysis showed a significant negative correlation between buffer capacity and DT (ρ = –0.444; p = 0.014) in the basketball players; as the salivary buffer capacity increased, the tooth decay index decreased (Table 5).

Correlation between changes in the salivary parameters due to the training session and the perceived exertion in swimmers and basketball players

The analysis of Spearman's correlation between differences in the pre- and post-training session salivary parameters and the RPE scores showed a significant negative correlation between V and RPE, and between Ca and RPE (ρ = –0.473; p = 0.008 and ρ = –0.427; p = 0.018, respectively) in the swimmers. No correlations were noticed in the group of basketball players (Table 6).

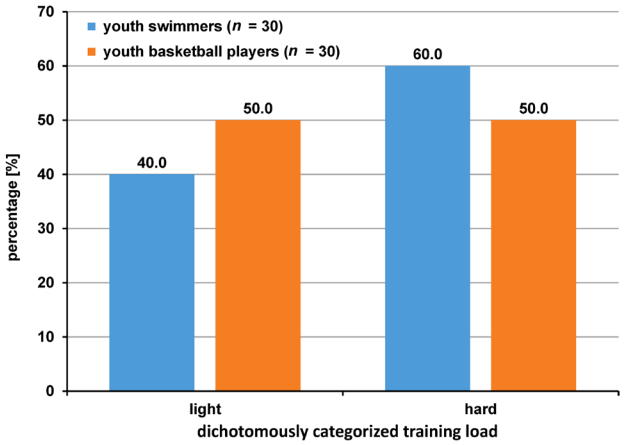


Fig. 3. Perceived exertion after the workout session in the studied groups

Logistic regression analysis

The bivariate logistic regression analysis conducted in the group of athletes showed only a significant effect of sAA on OHI-S (p = 0.054) in the swimmers and of pH on DMFT (p = 0.018) in the basketball players (Table 7).

The logistic regression analysis showed that of the salivary variables analyzed separately, only V, pH, buffer capacity, and Ca were associated with the perceived training load in youth swimmers. In contrast, in youth basketball players, none of the saliva variables were associated with the perceived training load (Table 8).

Table 6. Spearman's correlation between changes in the salivary parameters due to the training session and the rating of perceived exertion (RPE) scores in youth swimmers and basketball players

Group	Salivary parameters	RPE
Youth swimmers	V	ρ = –0.473 p = 0.008*
	pH	ρ = –0.214 p = 0.256
	buffer capacity	ρ = –0.255 p = 0.173
	Ca	ρ = –0.427 p = 0.018*
	sAA	ρ = 0.085 p = 0.655
	sPO	ρ = 0.093 p = 0.626
Youth basketball players	V	ρ = –0.010 p = 0.954
	pH	ρ = 0.078 p = 0.680
	buffer capacity	ρ = –0.052 p = 0.784
	Ca	ρ = –0.138 p = 0.468
	sAA	ρ = 0.065 p = 0.733
	sPO	ρ = 0.057 p = 0.764

* statistically significant (p < 0.05).

Table 7. Logistic regression analysis for the decayed, missing and filled teeth index (DMFT) and simplified oral hygiene index (OHI-S) scores as the dependent variables in youth swimmers and basketball players

Salivary parameters (independent variables)	Youth swimmers								Youth basketball players							
	DMFT				OHI-S				DMFT				OHI-S			
	b	lower 95% *CI*	upper 95% *CI*	*p*-value	*b*	lower 95% *CI*	upper 95% *CI*	*p*-value	*b*	lower 95% *CI*	upper 95% *CI*	*p*-value	*b*	lower 95% *CI*	upper 95% *CI*	*p*-value
V	0.070	−0.304	0.444	0.702	0.046	−0.311	0.403	0.792	0.433	−0.085	0.951	0.097	0.029	−1.151	1.209	0.960
pH	−0.316	−0.888	0.257	0.265	0.115	−0.432	0.662	0.668	0.317	0.058	0.575	0.018*	−0.044	−0.632	0.545	0.879
Buffer capacity	−0.003	−0.064	0.058	0.920	0.011	−0.048	0.069	0.710	−0.024	−0.054	0.006	0.115	0.016	−0.053	0.085	0.630
Ca	0.050	−0.095	0.195	0.484	0.031	−0.108	0.170	0.646	−0.015	−0.056	0.026	0.460	0.014	−0.080	0.108	0.762
sAA	0.002	−0.001	0.004	0.154	0.002	0.000	0.004	0.054*	0.001	0.000	0.002	0.169	0.002	−0.001	0.005	0.153
sPO	−0.226	−0.500	0.047	0.100	−0.128	−0.390	0.133	0.320	0.053	−0.167	0.272	0.624	−0.342	−0.842	0.158	0.171

b – logistic regression coefficient; *CI* – confidence interval; * statistically significant.

Table 8. Logistic regression analysis for the self-assessment of the training load as light or hard (according to Foster's RPE scale) as the dependent variable in youth swimmers and basketball players

Salivary parameters (independent variables)	Youth swimmers				Youth basketball players			
	b	lower 95% *CI*	upper 95% *CI*	*p*-value	*b*	lower 95% *CI*	upper 95% *CI*	*p*-value
V	0.070	−0.304	0.444	0.702	0.046	−0.311	0.403	0.792
pH	−0.316	−0.888	0.257	0.265	0.115	−0.432	0.662	0.668
Buffer capacity	−0.003	−0.064	0.058	0.920	0.011	−0.048	0.069	0.710
Ca	0.050	−0.095	0.195	0.484	0.031	−0.108	0.170	0.646
sAA	0.002	−0.001	0.004	0.154	0.002	0.000	0.004	0.054*
sPO	−0.226	−0.500	0.047	0.100	−0.128	−0.390	0.133	0.320

* statistically significant.

Discussion

The aim of the study was to assess the oral health status in terms of caries and oral hygiene in adolescents participating in various sports, and the effect of regular exercise on changes in selected saliva parameters in different sports environment. Significant changes in the oral ecosystem can be caused by lifestyle, oral hygiene, the diet, the frequency and duration of training, climatic conditions, and psychophysical stress.[3]

The most common diseases affecting the oral cavity of athletes are dental caries disease and periodontal disease, caused by plaque biofilm.[3] In a study by Needleman et al., examining young athletes, caries was found in 15–75% of subjects, and periodontitis in 15% of subjects; it was concluded that sports activities might be a risk factor for these oral diseases in athletes.[48]

In our study, caries prevalence in the study group of swimmers was 56.7%, which was lower as compared to the group of basketball players (66.7%). With regard to carious disease activity, as defined by the DT number, the study showed an average of 1.2 teeth in swimmers and 1.1 in basketball players. The MT number was 0 in both groups of subjects. On the other hand, the average FT number was 3.3 vs. 2.3, respectively. The average DMFT score in the 2 groups differed, and amounted to 4.5 in swimmers and 3.4 in basketball players. In Kaczmarek's study conducted among swimmers aged 13–43 years, the mean DMFT value was 4.71.[44] The author also reported the values of the components of the index, with DT = 1.42, MT = 0.19 and FT = 3.10, while the prevalence of caries in the group of swimmers was 54.8%.[44] A similar study assessing oral health was conducted by D'Ercole et al. in young swimmers (mean age: 12.5 years).[49] She found tooth decay in 38.24% of competitive swimmers, and the DMFT average score was 0.08.[49] In a study by Popa et al. on the prevalence of dental caries among children (mean age: 13 years) from the competitive soccer and field hockey groups, lower DMFT values were found in athletes as compared to the control group; soccer players had an average DMFT score of 2.10 and hockey players had an average DMFT score of 1.32, while the mean score in the control group was 3.4.[41] A number of studies show that for athletes, where good oral health is expected, the actual results do not confirm it. A review on the oral health of elite athletes found that caries was present in up to 75% of them.[43]

Oral diseases can be prevented by simple actions performed every day, like maintaining proper oral hygiene (effective plaque removal), among other things. Poor oral hygiene is a primary factor in the development of oral and systemic diseases.[3] In our study, all 3 oral hygiene indices (DI-S, CI-S and OHI-S) had significantly higher values for

swimmers than for basketball players ($p = 0.035$, $p = 0.014$ and $p = 0.006$, respectively). Oral hygiene analyzed with the use of OHI-S showed a value of 0.73 for swimmers and 0.34 for basketball players. The abovementioned study by Popa et al., who examined the impact of regular physical activity in young soccer and hockey players, found lower OHI-S values in these groups as compared to the control group; at the same time, the authors indicated a positive effect of competitive sports on oral hygiene in adolescents, in contrast to adults.[41] In another study,[49] the DI-S score in adolescents involved in swimming was similar to our own results (0.60 vs. 0.62).

It should be noted that some group sports require the use of mouthguards. Such mouth splints can be designed both for protective purposes and to deliver prophylactic substances to the tissues of the teeth, e.g., fluoride or chlorhexidine. However, they must be well designed, and replaced according to wear and tear, as worn or improperly made splints can damage the oral mucosa, the teeth and the periodontal tissues.[3]

Prolonged physical exertion, such as playing sports systematically, can reduce the unstimulated flow or amount of saliva, leading to dehydration and local dryness in the mouth, which considerably impairs the protective properties of saliva. This, in turn, may reinforce the negative impact of plaque biofilm, thereby increasing the cariogenic effect of dietary carbohydrates.[48] This finding was taken into account in the current study, and the salivary parameters were determined before and after the training in both groups of athletes. The literature shows that with reduced saliva secretion, and decreased pH and buffer capacity, the incidence of caries increases.[5] In both groups of young athletes studied (swimmers and basketball players), significantly reduced saliva secretion was observed after the training, i.e., by 22% in swimmers and by 26% in basketball players. It is likely that the greater decrease in saliva secretion in basketball players was related to the specific environment of the training performed, which is associated with greater exhaustion and dehydration. Decreased saliva secretion after exercise confirms the results of previous studies.[45,50] Different results were reported by Anttonen et al., who observed a significant increase in saliva secretion after exercise, which returned to the baseline value after 30 min.[51] Such a result was explained by the fact that the study was conducted at 10°C, which reduced dehydration.[51]

The pH of saliva is not a constant value, and under the influence of various factors, such as the rate of saliva secretion, a diurnal cycle, the diet, or autonomic nervous system or systemic diseases, it changes significantly. The pH of mixed saliva is 6.8. At night, when saliva secretion slows down, the pH value lowers to 6.2–6.5.[24] In our study, we observed a significant increase in the salivary pH, both after the swimming (by 2%) and basketball training (by 3%), a finding almost unanimously supported by other studies.[45,52] Different results were reported by Tanabe-Ikegawa et al., who observed a decrease in pH following a cycling exercise.[53] This would support the rationale that as salivary secretion decreases, pH also decreases, which is explained by changes in the salivary pH in association with the blood carbon dioxide (CO_2) levels. The blood concentration of CO_2 increases with athletic performance, resulting in a high concentration of CO_2 being transferred from the bloodstream to saliva, thereby causing a decrease in the salivary pH.[53] In our study, the regression model showed that pH could be a predictor of DMFT in basketball players. The buffer capacity of saliva and its ability to maintain a neutral pH is important for maintaining a healthy oral microbial composition, and thus it is responsible for maintaining adequate salivary homeostasis.[9] Our own research showed that both in swimmers and basketball players, buffer capacity decreased (by 10% vs. 12%, respectively). A significant decrease in buffer capacity was observed by Frese et al., who studied the effect of endurance training on the oral health of triathletes, in relation to tooth decay, among other things.[45] In our study, we found a negative correlation between buffer capacity and tooth decay in basketball players.

Micronutrients present in saliva are of great importance for the mineralization and maturation of the hard dental tissues. One of the elements necessary for the remineralization of tooth enamel is Ca.[5,54] Physical exercise of varying intensity and duration can alter the concentration of ions in saliva.[11] In our own study, a significant increase of 19% in Ca ions was observed in swimmers ($p = 0.001$), while a decrease of 11% was observed in basketball players. Similarly, a significant increase of Ca in saliva in male competitive swimmers exposed to a 2-hour swimming training session in a gas-chlorinated swimming pool was presented by Bretz and Carrilho.[50] In contrast, Chicharro et al. observed a decrease in Ca in athletes after intensive exercise on a cycloergometer.[55] The obtained results may be mainly related to the sport-specific environment.

Some saliva proteins can inhibit the adhesion of bacteria to oral surfaces. One of them is sAA, which is most abundant in saliva, as well as in the salivary membrane and plaque, and has a high affinity for oral bacteria; through binding to them, it inhibits their adhesion to various surfaces in the mouth.[9,12,24] Salivary alpha-amylase is involved in the formation of the glycoprotein complex in the salivary membrane, created on the surfaces of the teeth immediately after cleaning.[24] It also participates in the initial digestion of food, leading to the degradation of starch and glycogen into maltose to form more soluble compounds, which can dissolve in saliva.[9,12,24] Alpha-amylase is activated at pH above 6 and is inactivated in the acidic environment of the stomach.[9] In our study, the pre-training sAA levels were lower in swimmers as compared to basketball players. In both groups, the sAA levels increased after the training. Although the increase in basketball players as compared to swimmers was greater, the increase in swimmers was found to be statistically

significant (p = 0.028). The association of the enzyme level with exercise has been extensively investigated. Other studies conducted in both swimmers and basketball players have reported an increase in this enzyme in response to exercise.[33,56] In our study, we found a significant positive correlation between sAA and DI-S and OHI-S in swimmers. The regression model showed that sAA could be a predictor OHI-S in swimmers.

An increased respiration rate during exercise raises oxygen demand due to increased metabolism, which leads to increased production of harmful reactive oxygen species (ROS) and free radicals. Their negative effects can be neutralized by antioxidants. Salivary peroxidase is produced by the salivary gland cells and catalyzes the oxidation of thiocyanate ions by hydrogen peroxide (H_2O_2), thereby preventing the toxic accumulation of H_2O_2.[57] Sariri et al.[58] and Damirchi et al.[59,60] observed an increase in sPO activity in athletes immediately after intense treadmill running, which returned to baseline 1 h after exercise. Our data confirmed those results only in swimmers, as we found higher sPO levels after the swimming training session. In basketball players, on the other hand, the sPO levels decreased after the training session.

The assessment of training intensity in athletes can vary due to the environment of the sport played, as well as athletes' age and experience in competitive sports.[61] In a study by Barroso et al., examining swimming coaches and young swimmers, it was suggested that the more experienced the swimmers, the more accurate the RPE scores.[61] In addition, they observed that younger swimmers (11–12-year-olds) rated training intensity differently than coaches.[61] In the case of swimmers, exercise intensity may be experienced differently as compared to basketball players, as the swimming training takes place in a water environment, which alleviates the feeling of exhaustion. In contrast, in team sports, such as basketball, due to the situational nature of exercise, the subjective perception of intensity may be greater. Basketball requires frequent specific movements – from acceleration to deceleration, as well as changes in the direction of movement during the game. In a study by Lupo et al., the mean RPE score among 66 youth basketball players during the training session was 6.7 ±1.3.[31] The mean RPE values for our swimmers and basketball players after one training session were 4.96 ±1.75 and 5.13 ±1.91, respectively. Moreira et al., studying RPE in male basketball players using Foster's protocol, observed significantly higher scores after the official game as compared to the training session.[62] In our study, we found a negative correlation with the self-assessment of the exercise sensation according to Foster's RPE scale for both the salivary V and Ca level in swimmers. This finding meant that an increase in training severity correlated with a decrease in V and Ca. The regression model showed that the salivary V, pH, buffer capacity, and Ca level could be predictors of the effort rating among swimmers.

The project requires further research due to the wide variety of sports types. Its limitation is the size of sports club facilities and the number of members who can train in them. In addition, depending on the type of sport, gender separation is a limitation. Some sports divide teams by gender (basketball), while others are mixed (swimming). If the study continues, it will be necessary to take into account the disciplines by gender of the athletes, and in mixed disciplines, to survey an equal number of women and men.

If oral disease prevention and the promotion of oral health are accomplished through greater awareness of the impact of the diet and oral hygiene on the overall health of the body, the performance of athletes can be better and more stable, and prevention itself can become an important part of athlete care.

Conclusions

There were no significant differences in the DMFT values between the athletes, while significantly higher oral hygiene rates were found in swimmers. The exertion related to the swimming and basketball training caused a significant increase in pH and a decrease in the rate of saliva secretion. The level of sAA was positively correlated with DI-S and OHI-S in swimmers, and an increase in effort was negatively correlated with the salivary V and Ca levels among swimmers. However, the buffer capacity of saliva was negatively correlated with tooth decay in basketball players. The data suggests that the salivary V and pH, and the levels of Ca and sAA are associated with the swimming training session, whereas V and pH are also associated with the basketball training session. Both swimmers and basketball players rated the training as 'somehow hard' and 'hard'. The oral health status in athletes should be considered a priority during sports participation.

Ethics approval and consent to participate

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

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Digital recognition of color alteration in gingiva using a convolutional neural network

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Abstract

Background. Gingivitis is a common condition in individuals with inadequate oral hygiene. Although dental indices are widely used to assess periodontal status and educate patients, digital technologies have recently been incorporated into this field.

Objectives. The aim of the study was to provide an objective digital method to resolve the ambiguity between the first two scores of the gingival index (GI): absence of color alteration (score 0); and presence of color alteration in the gingiva (score 1), by designing a convolutional neural network (CNN) algorithm capable of detecting gingival color changes with high accuracy.

Material and methods. In this cross-sectional study, 10 CNN models were developed and trained to distinguish between normal and abnormal gingival color. A total of 6,660 augmented, pre-classified frontal-view images of the patients' gingiva were included. The dataset was divided into a training set (4,640 images; 70%) to train the CNN models and a test set (2,020 images; 30%) to test them. All images were previously classified by periodontists as representing either normal (0) or abnormal (1) gingival color. During training, the CNN models learned to classify gingival color based on these reference labels. Model performance was subsequently evaluated in the test phase by comparing the models' predictions with the reference diagnoses provided by periodontists.

Results. To evaluate inter-rater reliability, model performance was examined using Cohen's kappa coefficient and classification accuracy relative to the reference diagnoses established by the periodontists. Model 4 demonstrated excellent inter-rater reliability ($\kappa = 1.00$) and achieved 99.9% accuracy, followed by Model 8 (99.7%) and Model 5 (99.3%). Models 7 and 3 showed lower accuracies of 81.1% and 88.1%, respectively. All models demonstrated statistically significant agreement with the reference diagnoses ($p < 0.001$).

Conclusions. Differentiation between GI scores of 0 and 1 using CNNs provides an objective method for detecting gingival color alterations. This approach has potential for improving patients' awareness of periodontal health, enhancing motivation to maintain oral hygiene, and encouraging dental visits.

Keywords: artificial intelligence, convolutional neural network, gingivitis, digital dentistry, gingival index

Highlights

- A convolutional neural network was trained to detect gingival color changes and distinguish normal from abnormal gingival color.
- The artificial intelligence (AI) model focused on gingival color assessment and avoided providing comprehensive periodontal diagnosis.
- The convolutional neural network demonstrated high diagnostic accuracy in identifying gingival color changes when compared with the reference diagnoses made by periodontists.
- The findings support further validation to improve AI performance before clinical implementation.

Introduction

Periodontal diseases are the 11th most prevalent group of diseases worldwide, affecting an estimated 20–50% of the global population.[1] Gingivitis is an inflammatory condition induced by bacterial plaque and/or bacterial toxins that is confined to the soft tissues of the periodontium. It is a mild and reversible form of periodontal disease that may progress, if left untreated, to periodontitis,[2,3] an inflammatory disease affecting both soft and hard tissues of the tooth-supporting apparatus and resulting in irreversible periodontal attachment loss.[4]

Clinical indices are standardized objective methods designed to evaluate dental and periodontal conditions.[5] Periodontal indices have evolved considerably over the years, from early forms such as Russel's periodontal index (1956) to more sophisticated and relatively recent indices, including the Genetic Susceptibility Index (GSI) introduced in 2007. The purpose of periodontal indices is to improve the diagnosis and assessment of periodontal diseases as well as to facilitate the development of appropriate preventive and treatment strategies.[6]

Some periodontal indices are designed to evaluate specific clinical conditions, such as the gingival index (GI),[7] whereas others assess oral hygiene status, such as the plaque index (PI).[5] More comprehensive indices have been developed to evaluate multiple periodontal conditions and estimate community treatment needs, such as the community periodontal index of treatment needs (CPITN), introduced by the World Health Organization (WHO) and assessed using the WHO periodontal probe.[8] The oral hygiene index (OHI) is used to examine overall oral hygiene status using numerical scores ranging from 0 to 3. It is simple, reproducible and reliable; however, it has some limitations. The index does not consider plaque and does not provide a substitution for missing teeth as it divides the oral cavity into sextants. Other indices focus on measuring periodontal destruction, including probing pocket depth (PPD) and clinical attachment loss (CAL). Although these indices provide valuable quantitative and qualitative information, they are more technique-sensitive, require greater examiner experience, and may show lower reproducibility than simpler clinical indices.

A good periodontal index should satisfy several criteria: (1) clarity, allowing easy interpretation and facilitating clinical decision-making; (2) simplicity; (3) objectivity, minimizing examiner-dependent variation; (4) validity, ensuring that the index measures the intended clinical condition; (5) reliability and reproducibility, providing consistent results when the clinical condition remains unchanged; (6) quantifiability, allowing statistical analysis; (7) sensitivity, enabling the detection of subtle clinical changes; and (8) acceptability, ensuring that the examination is well tolerated by patients. The strengths and limitations of periodontal indices are determined by the extent to which they satisfy these criteria.[9]

The main purpose of the GI is to assess the gingival condition, including the quality of the gingiva, and to identify the location of the lesion (buccal, mesial, distal, and lingual surfaces). The GI is non-invasive, atraumatic and easy to perform. However, it is inherently subjective and does not assess PPD, degree of bone loss or other quantitative indicators of the periodontium.[7]

The integration of digital technologies into dentistry has improved both clinical examination and treatment procedures.[10] Devices such as digital X-ray sensors, computed tomography (CT) scanners, microscopes, and loupes[11] have enhanced diagnostic precision.[12] More recently, smartphone applications have emerged as adjunctive tools in the process.[13]

Pain is one of the primary reasons for patients to seek dental care.[14,15] However, pain is generally absent during early stages of gingival disease, including gingivitis and peri-implant mucositis.[16] Consequently, particularly in countries without mandatory dental insurance systems[17] or among individuals lacking dental insurance coverage, periodontal disease may remain unnoticed until more advanced stages develop.[18]

Smartphone applications have transformed many aspects of everyday life, including advertising,[19] office applications, tools, and healthcare.[20] In dentistry, mobile applications have the potential to improve dental awareness and bridge the gap between patients and dentists.[21] Because many individuals postpone routine dental visits in the absence of pain or other obvious symptoms, user-friendly mobile applications capable of providing an initial

assessment of gingival and periodontal status may encourage earlier professional evaluation.

However, digital diagnostic systems should be regarded as decision-support tools rather than replacements for professional clinical judgement.[22] The final diagnosis and treatment decision should always be made by qualified dental professionals.[23]

Online information and self-assessment applications may provide misleading interpretations of oral conditions,[24] potentially leading patients to overestimate disease severity or seek unnecessary treatment.[25]

Pain in the oral cavity or dental pain has multiple etiologies, with pulpitis and periodontal pain representing common causes.[26,27] However, patients' perception of pain, dissatisfaction and awareness is influenced by psychological factors.[28] The digital system developed in the present study was designed to provide users with a preliminary assessment of whether professional periodontal evaluation is advisable. At the same time, the system aims to reduce the ambiguity between GI scores of 0 and 1 without attempting to identify the exact location of inflammation or an opinion about the teeth's color. This approach preserves the essential role of the dentist in establishing the final diagnosis and treatment plan while reducing the likelihood of inappropriate self-diagnosis.

Artificial intelligence (AI) has become increasingly integrated into different fields,[29] especially medicine.[30] AI-based methods support decision-making and facilitate solving complex real-world problems.[31] Convolutional neural networks (CNNs) are artificial neural networks[32,33] designed for image analysis. Convolutional neural networks play an important role in computer-aided diagnosis (CAD)[34] by accurately detecting objects and classifying them by processing the patient's raw data.[35] In the present study, the CNN performed 2 principal tasks: object detection, which localizes the region of interest within an image; and image classification, which assigns the detected region to a predefined class using supervised learning.[36,37]

The aim of this study was to develop and evaluate a CNN capable of distinguishing between normal and abnormal gingival colors using standardized frontal photographs that had been previously annotated by experienced periodontists.

Material and methods

The study was conducted in accordance with the principles of the Declaration of Helsinki.[38] Written informed consent was obtained from all participants before enrollment. The study protocol was reviewed and approved by the Ethics Committee of the College of Dentistry, University of Al-Mashreq, Baghdad, Iraq (order No. 245, 2022).

Patient selection, grouping and exclusion criteria

Patients attending the Department of Periodontology at the College of Dentistry, primary healthcare centers, and specialized dental centers in Baghdad, Iraq, between September 2021 and October 2022 were screened for eligibility. Clinical examinations were performed by a team of 3 periodontists using direct visual inspection and dental mirrors to distinguish healthy gingiva from gingivitis. The final diagnosis was established after discussion and consensus among the 3 examiners. Demographic characteristics, including race and sex were not considered as selection criteria.

The exclusion criteria were as follows: mentally challenged patients; terminally ill patients; children aged <12 years; patients with traumatic gingival wounds or non-inflammatory gingival bleeding; and patients with drug-induced gingivitis.

To maximize the diversity of gingival color patterns at different stages of gingivitis, a large image dataset was collected. A larger and more diverse dataset enables the CNN to learn a wider range of normal and abnormal gingival color variations, thereby improving its ability to recognize subtle color changes in previously unseen images. Furthermore, including participants of different ages, sexes and ethnic backgrounds increases the reliability of the model while reducing the risk of overfitting and over-specialization.[39]

The image dataset was divided into training and test sets using an approximately 70:30 split (4,640 and 2,020 images, respectively), which is widely accepted for machine learning applications while allowing flexibility based on the complexity, size and type of the dataset.[40]

The training dataset consisted of 4,640 augmented images:

- group A – 1,950 images of clinically healthy gingiva;
- group B – 2,690 images of abnormally colored gingiva.

The test dataset consisted of 2,020 images:

- group A: 850 images of clinically healthy gingiva;
- group B: 1,170 images of abnormally colored gingiva.

After model training, all images from the test dataset were analyzed using the trained CNN models. The model predictions were compared with the reference diagnoses established by the periodontists to determine classification accuracy and reliability (Fig. 1).

Materials

The following equipment and software were used during the study:

- digital camera (Nikon D3200; Nikon Corporation, Tokyo, Japan);
- plastic lip retractor;
- dental mirror and WHO/CPITN periodontal probe (Hu-Friedy, Chicago, USA);

- cotton pellets and patient towels;
- class B autoclave sterilizer (Woson, Ningbo, China);
- 70% alcohol for cold sterilization;
- laptop computer (MSI GL63 8RD; MSI, New Taipei City, Taiwan) equipped with an Intel® Core™ i7-8750H processor, 16 GB RAM, and an NVIDIA GP107M (GeForce GTX 1050 Ti Mobile)/Mesa Intel® UHD Graphics 630 (CFL GT2) graphics card;
- Python3 programming language with TensorFlow 2.8.0.

Gingival index

The gingival index was used to examine and classify gingival condition. The present study focused primarily on the first two GI scores. The index was first introduced by Löe,[41] with the scores defined as follows:

- 0 – normal gingiva with no discoloration or redness and no bleeding on probing;
- 1 – reddened gingiva characterized by changes in color and/or texture without bleeding on probing;
- 2 – redness, edema, glazing, and slight bleeding on probing;
- 3 – marked redness and edema/hypertrophy, ulceration, amorphous/continuous bleeding on probing, or spontaneous bleeding.

Examiner calibration

To improve diagnostic consistency, the 3 periodontists underwent examiner calibration before the study. Clinical assessments were performed independently and in a blinded manner. The final diagnosis for each participant was accepted only when at least 2 of the 3 examiners reached agreement regarding the GI score.

Image acquisition and sample classification

Frontal intraoral photographs were taken using a digital camera (Nikon D3200; Nikon Corporation) fitted with an 18–55 mm lens. A plastic lip retractor was used to expose the gingiva, and the camera was focused on the contact point between the upper and lower central incisors. No chair light or camera flash was used while taking the photographs in order to preserve the natural appearance of gingival color. All patients were seated at the same location in the examination room. Illumination was provided exclusively by natural daylight entering through a wide window (2 m × 1 m) located 2 m in front of the participant. Photographs were acquired between 9 am and 11 am. For standardization, the camera was mounted on a tripod at a fixed distance of 30 cm from the patient's face, and no background plate was used.

Following image acquisition, all photographs were transferred to a computer and divided into 2 classes based on the periodontists' decision: normal gingival color (group A, GI score 0); or abnormal gingival color (group B, GI score ≥1).

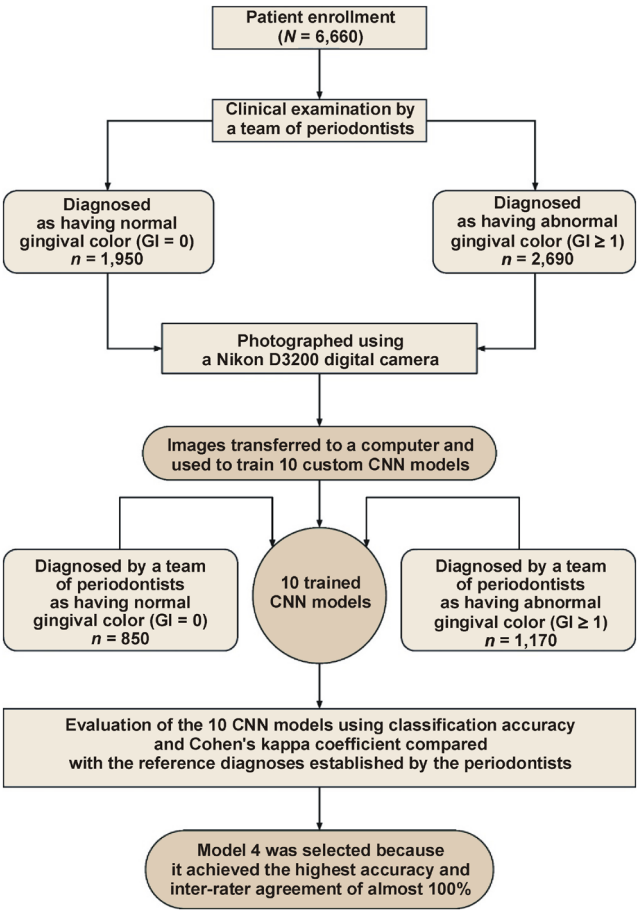


Fig. 1. Flowchart of the study design

CNN – convolutional neural network.

Image preprocessing

Raw images were resized to 1,024 × 768 pixels and stored in JPEG format using the RGB color space. Because the objective of the study was to analyze gingival color, the surrounding area (jaw, nose, lips, background) was excluded from further analysis. The gingival region was annotated using the LabelImg image annotation software.[42] Each image was manually labeled with a bounding box encompassing the gingival tissues. Subsequently, the gingival regions were detected and extracted using the Single Shot MultiBox Detector (SSD), a CNN-based model specifically developed for efficient object detection. The SSD model identified the gingival region and produced cropped images for subsequent classification.

Gingival detection using the Single Shot MultiBox Detector

The primary challenge of this research was the accurate extraction of the gingival region from images. Object detection techniques enable automatic localization of structures of interest within digital images. The SSD is a superior CNN-based method employed in object detection with lower computational demands. The Single Shot MultiBox

Detector processes an image in a single pass, using feature maps at various scales to predict object boundaries and categories. During training, the model requires only the input images and their corresponding ground-truth boxes for each object, simplifying the learning process while maintaining high detection accuracy. Consequently, SSD was selected for application in the present study (Fig. 2,3).

Image classification

Image classification involves assigning input images to predefined categories or classes using machine learning algorithms.[43] In the present study, the CNN was trained to classify gingival images into one of the 2 categories: normal gingival color or abnormal gingival color. When presented with new data, the trained network analyzed the learned image features and assigned each image the most probable class based on its similarity to the training data. This ability to automatically extract relevant image features is one of the principal advantages of CNNs and contributes to their superior performance.

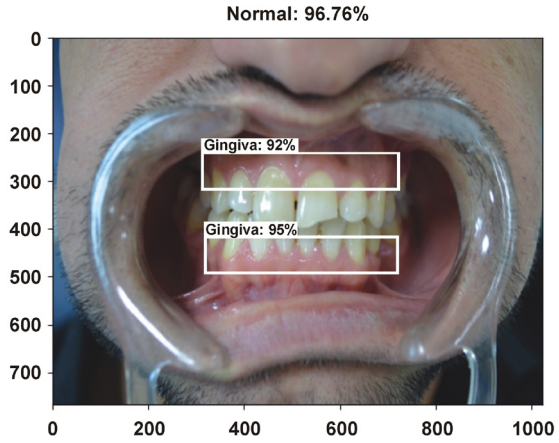


Fig. 2. Gingiva classified by the convolutional neural network (CNN) as having normal gingival color

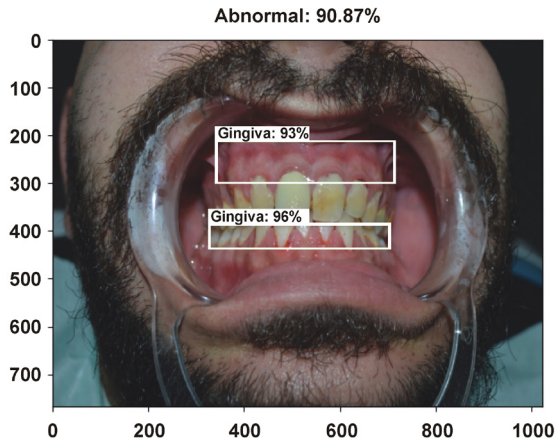


Fig. 3. Gingiva classified by the convolutional neural network (CNN) as having abnormal gingival color

Convolutional neural network

A convolutional neural network, also referred to as ConvNet, is a type of artificial neural network (ANN) specifically designed for image analysis. Unlike traditional fully-connected (FC) neural networks, CNNs employ a deep feed-forward structure and exhibit superior generalization performance. They can learn complex, abstract features of objects, particularly those that involve spatial data, and recognize them with high efficiency. A CNN consists of multiple layers, each serving a specific function (Fig. 4). The network begins with an input layer that receives the image data. In our study, the input images had dimensions of 224 px × 224 px × 3 px. The input layer was connected to a convolutional layer, which was responsible for extracting relevant image features, including color, texture and softness of the gingiva. Pooling layers were subsequently applied to reduce the number of samples. Furthermore, the FC layer was used to classify the features generated from the pooling layer. Finally, the output layer generated a prediction, classifying each image as either normal gingiva (0) or abnormal gingiva (1).

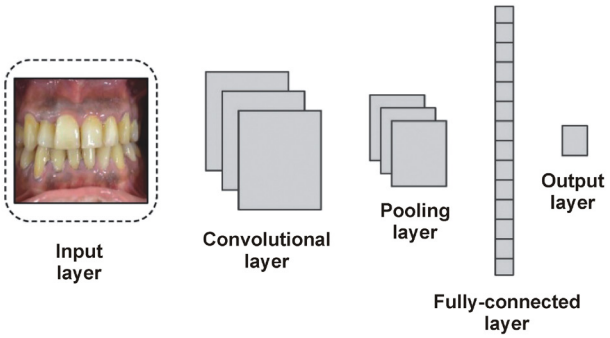


Fig. 4. Architecture of the convolutional neural network (CNN)

Development and training of the CNN models

Custom CNN models were developed specifically for the present study rather than employing standard deep learning models[44] such as Visual Geometry Group (VGG) or Residual Network (ResNet), which contain a large number of trainable parameters and require longer training time.[45] To optimize computational efficiency, 10 custom CNN models were designed and evaluated using the present dataset. These models provide maximum accuracy in the shortest amount of time and with the least number of parameters.

The dataset was divided into training (70%) and test (30%) subsets. The training process was iterated multiple times to facilitate learning by adjusting the weight and enabling the system to distinguish between normal and abnormal cases. The training was repeated several times until the model reached a state of stability.

Prevention of overfitting and underfitting

Several strategies were implemented to reduce the risk of overfitting during model development. First, the CNNs were trained using a relatively large dataset comprising approx. 6,660 images, which represented a diverse sample size. Second, data augmentation techniques were applied to increase the diversity of the training set and improve model generalizability. Finally, model performance was continuously monitored on both the training and test datasets. The alignment between the metrics indicated that the models showed no evidence of substantial overfitting or underfitting and maintained strong predictive capabilities of the test dataset.

Model evaluation

After completion of the training, the CNN models were tested using a dataset of 2,020 images. The predicted classifications were compared with the reference diagnoses established by periodontists at the Department of Periodontology, using the following formula:

$$accuracy = \frac{number\ of\ correct\ predictions}{total\ number\ of\ predictions}$$

Statistical analysis

Statistical analyses were performed using the IBM SPSS Statistics for Windows software, v. 26.0 (IBM Corp., Armonk, USA). Cohen's kappa coefficient was calculated to evaluate the agreement between the CNN classifications and the reference diagnoses (inter-rater reliability/agreement test).

According to Cohen, kappa values are interpreted as follows[46]:

- ≤0.00: no agreement;
- 0.01–0.20: poor to slight agreement;
- 0.21–0.40: fair agreement;
- 0.41–0.60: moderate agreement;
- 0.61–0.80: substantial agreement;
- 0.81–1.00: almost perfect agreement.

Results

Descriptive statistics of the training dataset

A total of 4,640 images were included in the training dataset and used to train the CNN models according to the reference diagnoses established by the 3 periodontists. Of these, 1,950 images were classified as healthy gingiva (GI score 0), whereas the remaining 2,690 images were classified as abnormal gingiva (GI score ≥1). The standard deviation of the test dataset was 0.493654. The middle quartile (Q2) value was 1.00, and the interquartile range (*IQR*) was 1.00 (Table 1).

Table 1. Descriptive statistics of the training dataset

Variable		Result
Valid samples, *n*		4,640
Missing samples, *n*		0
SD		0.49
Quartile	Q1	0.00
	Q2	1.00
	Q3	1.00

SD – standard deviation.

Descriptive statistics of the test dataset

The test dataset consisted of 2,020 images used to evaluate the trained CNN models. Based on the reference diagnoses established by the periodontists, 850 images were classified as normal gingiva and 1,170 images as abnormal gingiva (dark or abnormal color). The standard deviation of the test dataset was 0.493808. The middle quartile was 1.00, and the *IQR* was 1.00 (Table 2).

Table 2. Descriptive statistics of the test dataset

Variable		Result
Valid samples, *n*		2,020
SD		0.49
Quartile	Q1	0.00
	Q2	1.00
	Q3	1.00

Performance of the CNN models

The test dataset comprising 2,020 images was evaluated using 10 different CNN models. Then, the accuracy was determined, and the interrater reliability of every model was tested by calculating Cohen's kappa coefficient (Fig. 5).

The classification accuracy, Cohen's kappa coefficient and corresponding *p*-values for each model are presented in Table 3. Among the evaluated models, Model 4 achieved the highest accuracy (99.9%). The lowest accuracy was observed for Model 7 (81.1%), whereas Model 3 achieved an accuracy of 88.1%. All remaining models demonstrated classification accuracy exceeding 90%.

Model 4 exhibited perfect agreement with the reference diagnoses ($\kappa = 1.00$; $p < 0.001$). The remaining models

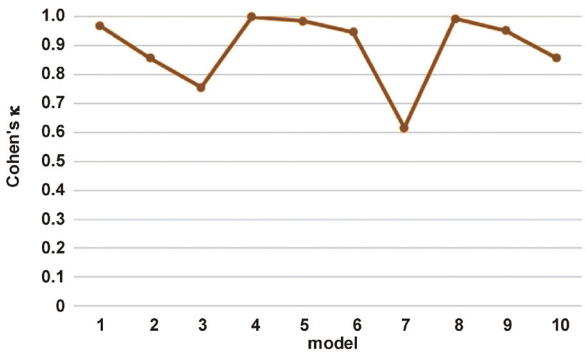


Fig. 5. Cohen's kappa coefficients for the agreement between the 10 convolutional neural network (CNN) models and the reference diagnoses established by the periodontists

achieved Cohen's kappa values ranging from 0.86 to 0.99, indicating almost perfect agreement with the periodontists' classifications. Model 7 was an exception (κ = 0.62), lying on the lower border of what is known as substantial agreement. Model 3 achieved a kappa value of 0.76, which also indicates substantial agreement. Despite their comparatively lower performance, both models remained statistically significant ($p < 0.001$).

Table 3. Classification accuracy, Cohen's kappa coefficients and *p*-values for the 10 convolutional neural network (CNN) models

Model	Accuracy	Cohen's κ	*p*-value
1	98.5%	0.97	<0.001*
2	93.1%	0.86	<0.001*
3	88.1%	0.76	<0.001*
4	99.99%	1.00	<0.001*
5	99.3%	0.99	<0.001*
6	97.5%	0.95	<0.001*
7	81.1%	0.62	<0.001*
8	99.7%	0.99	<0.001*
9	97.8%	0.95	<0.001*
10	93.1%	0.86	<0.001*

* statistically significant ($p < 0.05$).

Figures 6 and 7 show the comparison between the training curve pattern (expected pattern) and the test curve pattern during model development. Among the evaluated models, Model 4 demonstrated an almost perfect simulation of the expected curve pattern.

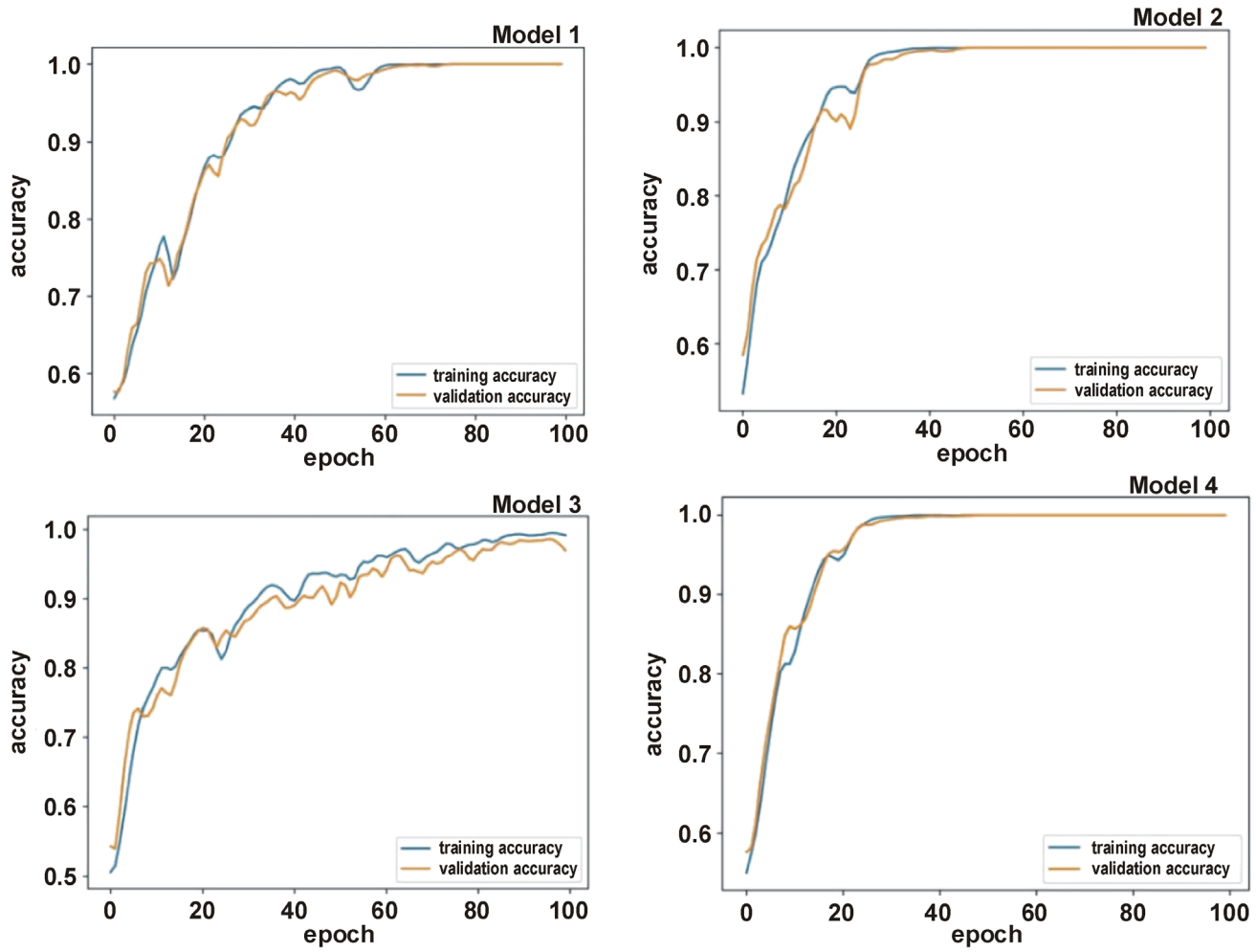


Fig. 6. Training and validation accuracy curves for Models 1–4

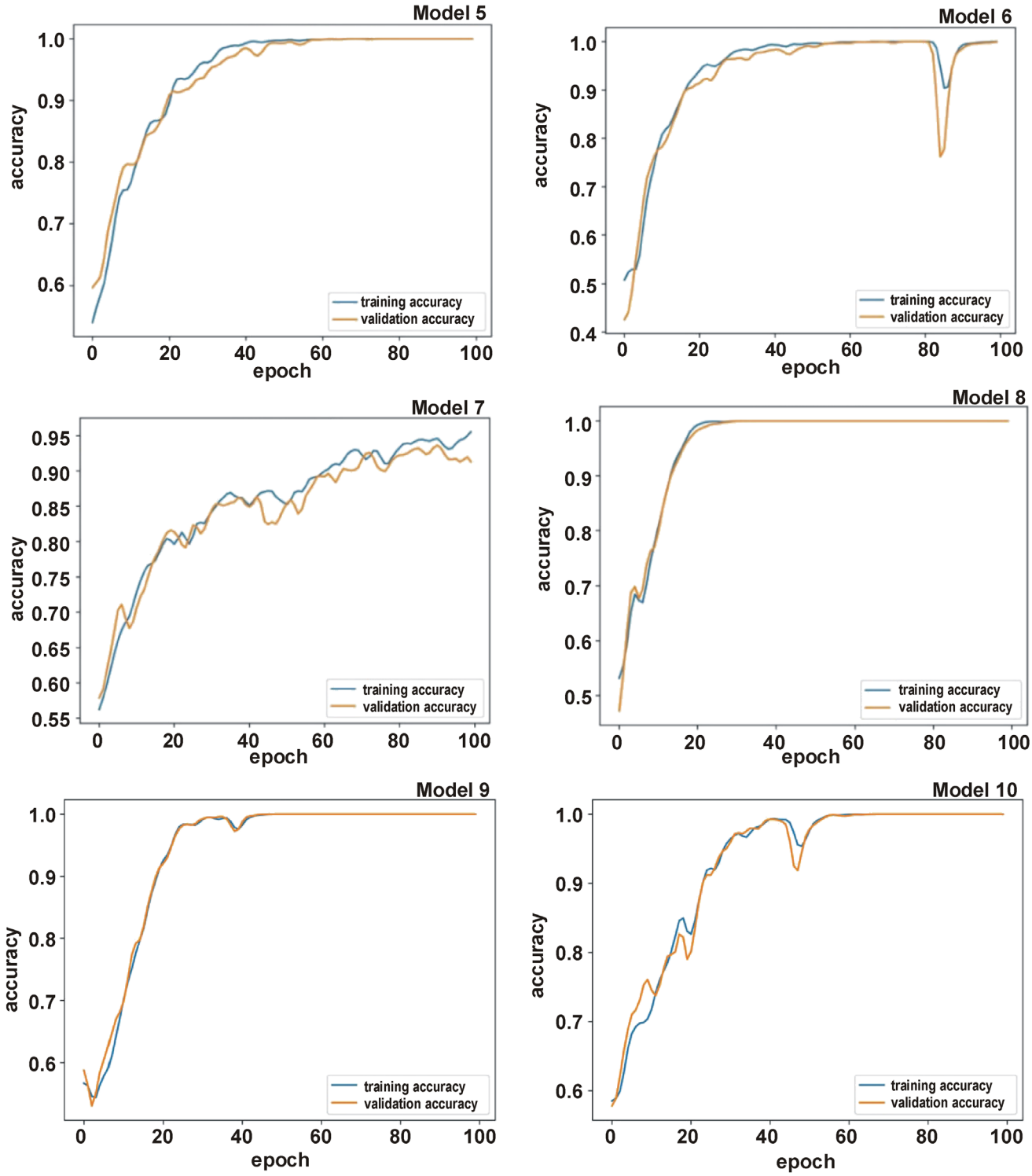


Fig. 7. Training and validation accuracy curves for Models 5–10

Discussion

The integration of digital technologies into dental diagnosis and treatment planning has expanded considerably over the past 2 decades. Applications including computer-assisted interpretation of diagnostic images, three-dimensional treatment planning, face-driven orthodontics, digital surgical planning, prosthetic design, and three-dimensional (3D) printing have transformed many aspects of clinical dentistry. These technologies aim to improve diagnostic precision and treatment outcomes by providing objective results that complement conventional clinical assessment.[47–49] Although experienced clinicians can interpret a wide range of clinical presentations, diagnostic performance may be influenced by stress and environmental factors.[50] Furthermore, many medical

conditions present with overlapping clinical features,[51] making the distinction between normal and abnormal findings challenging.[52] As previously mentioned, factors such as long working hours, stress and fatigue, which are common in the medical field, may lead to a decline in concentration, resulting in what is known as human error in diagnosis.[53] Consequently, AI has become increasingly incorporated in this field for the detection of eye diseases, breast cancer, fetal anomalies, and numerous other medical conditions.[54]

Assessment of gingival color remains one of the most subjective components of the GI, particularly when differentiating between scores of 0 and 1, where only slight changes in color may be present.[41] The human eye is a subjective tool. Unless it is calibrated by other examiners, or accompanied by the use of adjunctive devices,[55,56] or, as we present in this article, digital methods, it is not objective.[48]

Convolutional neural networks are inspired by the mechanism of the visual cortex in animals. The manner in which animals perceive images is distinct from that of humans. In order to facilitate the retention and subsequent recognition of a particular image, it is necessary for animals to be exposed to multiple images and augmented photographs.[57]

Several CNN models were tested to detect gingival color alterations. The decisive factor in preferring one model over another is the trial-and-error process,[58] since there is no objective method to determine the best CNN model for different inputs and experiments.[59]

Raw images were imported into the system and then subjected to augmentation, including horizontal and vertical flipping, random cropping, and several variations in color or intensity. This led to the multiplication of the number of images.[59] The increased dataset obtained by applying such techniques improved the accuracy of the models. This method of image processing and augmentation was successfully adopted by Thaha et al. to detect brain tumors in magnetic resonance imaging (MRI) scans.[60]

Even though increasing the number of images in the model (up to thousands of images) can be useful, care must be taken to not overfit the model.[61]

Errors occur for several reasons, including a lack of data, the type of model used, or high correlations among the classes (normal and abnormal), which may be misdiagnosed by an expert.[62]

Ten CNN models were constructed based on the research problem. Model 4 (Fig. 6) was the simplest model, with the fewest parameters and layers, and achieved excellent accuracy.[63] The development of custom CNN models for gingival disease detection is just one example of how advanced machine-learning techniques can be applied in the medical field.[29,62]

In the present study, we followed a slightly different method of utilizing technology and software programming in dental practice. Unlike other researchers who have carried out extensive work to create software that can be used for self-diagnosis and even provide advice on self-treatment and suggestions for preventive actions (e.g., Liang et al.),[63,64] we tried to simplify the procedure and develop software that is limited to providing a primary idea or an initial indication for patients to have their oral cavity examined by a specialist. Additionally, the tool provides an objective method for periodontists and general dental practitioners to distinguish between GI scores of 0 and 1.

The reason for the development of this tool was to avoid giving the patient a preconceived mindset[61] that might cast a negative shadow on the examination procedure and the treatment plan to be established by the dentist. Otherwise, the patient may have already made a self-diagnosis and may wish to formulate a treatment plan independently.

An older research model was adopted by Seshan and Shwetha and tested on a small number of patients.[65] The researchers used Serif PhotoPlus 6 software (Serif Ltd, Nottingham, UK), which relied on counting pixels in cropped areas of red, inflamed or bleeding gingiva. The comparison was performed between the same areas in the same patient after proper plaque control, using a red disc as a reference.[65] The work model utilized in the present study differed, as we relied on a trained software model to digitally analyze images of the entire facial gingiva without using a foreign body as a reference. This method enabled a primary assessment of the gingival status.

Limitations

The present study has several limitations.

First, only frontal intraoral photographs were analyzed. Future studies should investigate whether incorporating additional views and angles improves classification performance.

Second, image acquisition was performed using a single camera system. Future investigations should evaluate the performance using smartphone cameras, to better reflect real-world applications.

Third, all photographs were obtained under standardized lighting conditions to increase reliability and generalizability. Future studies should assess model performance under a broader range of lighting conditions, including both indoor and outdoor environments.

Finally, the proposed model evaluated only 1 clinical feature, namely gingival color alteration. Future research should integrate additional variables used not only by patients but also by dentists themselves, such as vertical/horizontal radiological bone resorption level and the presence or absence of clinical attachment loss.

Conclusions

The results of the present study demonstrate that CNN software models can be trained to accurately detect slight

changes in gingival color. These models have the potential to be incorporated into patient-oriented applications that promote oral health awareness and encourage timely dental visits before the progression of gingivitis.

Ethics approval and consent to participate

The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment. The study protocol was reviewed and approved by the Ethics Committee of the College of Dentistry, University of Al-Mashreq, Baghdad, Iraq (order No. 245, 2022).

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Original paper

Systemic inflammatory acute-phase response following palatal graft harvesting in rats

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Abstract

Background. Surgical injury triggers a stereotypical cascade of responses known as the systemic inflammatory response (SIR). To the best of our knowledge, the SIR following palatal graft harvesting has not been previously investigated.

Objectives. The aim of the study was to examine the magnitude of the SIR following palatal graft harvesting in Wistar rats by assessing serum levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-18, peripheral white blood cell (WBC) counts (differential and absolute counts of lymphocytes, neutrophils, monocytes, eosinophils, and basophils); and anemia-related parameters (red blood cell (RBC) count, hemoglobin (Hgb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)).

Material and methods. A total of 48 male Wistar rats were included in the study. A 4.2-mm palatal wound was created using a biopsy punch in the middle of the palate. Blood samples were collected before surgery and at 24, 48, 72, and 96 h postoperatively. Serum levels of TNF-α, IL-6 and IL-18 were measured using enzyme-linked immunosorbent assay (ELISA), and complete blood counts were analyzed with an automated blood cell counter on the day of sacrifice.

Results. TNF-α levels peaked on day 1, IL-18 on day 2, and IL-6 on day 3. All cytokine levels returned to baseline by day 4. A significant 40% decrease in WBC count was observed on day 1, primarily due to a 47% reduction in lymphocyte count despite a significant 50% increase in neutrophil count. Red blood cell count, Hgb and HCT decreased significantly by 25%, 23% and 23%, respectively, with the greatest reductions observed on days 1, 1 and 2. All values returned to baseline by day 4.

Conclusions. The dynamics of SIR following palatal graft harvesting resemble those observed after other minor surgical procedures. The findings support the classification of periodontal and oral surgery as minor surgery. Changes in blood cell counts and cytokine levels may help identify situations requiring closer monitoring to promote optimal wound healing.

Keywords: IL-6, TNF-α, IL-18, hemoglobin, systemic immune response

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Highlights

- Systemic inflammatory response after palatal graft harvesting resembles the response observed after other minor surgical procedures.
- The findings support classifying periodontal and oral surgery as minor surgical procedures.
- Changes in blood cell counts and cytokine levels may help identify patients requiring closer monitoring to promote optimal wound healing.

Introduction

Palatal tissue harvesting is routinely performed in oral, periodontal and systemic surgery. The most common drawback of this procedure is postoperative patient morbidity, although other complications may also occur. Therefore, elucidating the local and systemic factors, biological agents and genes involved in palatal wound healing is essential.[1]

Surgical injury triggers a well-defined cascade of systemic inflammatory responses.[2] This response is characterized by increased production of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α, interleukin (IL)-18, and, in particular, IL-6.[3] Together, these cytokines constitute a complex signaling system referred to as the systemic inflammatory response (SIR).[4] In the oral cavity, changes in the levels of inflammatory mediators involved in the SIR are induced not only by surgical procedures but also by prosthetic rehabilitation and its chronic inflammatory effects on the peri-implant soft tissues.[5–11] The systemic inflammatory response promotes skeletal muscle catabolism to provide energy and substrates for the liver, maintains fluid and cardiovascular homeostasis, and supports wound healing at the surgical site. The magnitude of the increase in these markers is considered to reflect the intensity of the SIR.[2]

The cellular hallmark of the SIR is the response of white blood cells (WBCs), including lymphocytes, neutrophils, monocytes, eosinophils, and basophils, each type having a distinct role in the immune response. The primary function of the immune system is to protect the host against pathogenic microorganisms and foreign substances.[12] Following surgery, which represents a sterile injury, this response is reflected by acute changes in WBC counts despite the absence of microorganisms or foreign material. These changes are thought to be triggered by either physical or psychological stress. Accordingly, WBC parameters have been widely used as indicators of the physiological stress response.[13]

Postoperative acute-phase anemia has also been reported following surgery. Surgical anemia is usually subclinical and associated with iron deficiency, and it may persist for up to 8 weeks.[14]

A better understanding of the SIR associated with oral surgical procedures may improve postoperative patient management. Several strategies have been proposed. Refinement of surgical techniques, particularly the adoption of minimally invasive approaches, may reduce the inflammatory response. Laparoscopic surgery, for example, has been associated with faster postoperative recovery. Nutritional interventions, including glutamine, arginine and omega-3 fatty acid supplementation, may also improve outcomes. In addition, anabolic steroids and insulin infusions, with or without glucose supplementation, may also minimize muscle wasting. Maintenance of normothermia has also been suggested as a strategy to reduce the SIR.[15]

The rat is a well-established experimental model for investigating systemic responses to surgical injury.[16] Metabolic studies have demonstrated that surgery induces skeletal muscle catabolism as part of a generalized adaptive response aimed at promoting survival and tissue repair. Because injury reduces activity and food intake, the body relies on its internal sources of energy (protein and fat) to enable wound healing and physiological maintenance.

The palate is the preferred donor site for harvesting autogenous grafts, and the development of strategies to minimize postsurgical morbidity remains of considerable clinical interest.[17] Therefore, the present study aimed to evaluate the magnitude of the SIR following palatal graft harvesting in Wistar rats by assessing serum levels of TNF-α, IL-6 and IL-18, peripheral WBC counts and anemia-related parameters.

Material and methods

The study protocol was approved by the Ethics and Institutional Animal Care and Use Committee of Tel Aviv University, Israel (approval No. TAU – MD–IL-2402-109-3; February 1, 2024). All animals received humane care throughout the study. The experimental design complied with the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines.[18] A total of 48 male Wistar rats were randomly allocated to 4 equal groups (n = 12 per group). The groups were categorized according to the postoperative day on which the animals were euthanized (days 1–4).

General anesthesia was induced on the day of surgery by intraperitoneal injection of 10% ketamine (90 mg/kg; Kepro, Deventer, the Netherlands) and 2% xylazine (10 mg/kg; Medical Market, Tel Izhak, Israel).

Immediately before surgery (day 0), baseline blood samples were collected from all animals by independent laboratory personnel.

All surgical procedures were performed by the same experienced operator (LC). Following blood collection, a 4.2-mm-diameter circular excisional wound was created in the center of the palatal mucosa using a tissue punch (MIS Implants Technologies, Bar-Lev Industrial Park, Israel). This procedure resulted in a circular area of denuded bone left for secondary healing. Gentle pressure was applied with gauze until hemostasis was achieved.

A 2-h postoperative feeding break was advocated to minimize mechanical trauma to the surgical site. Soft food was then provided for the following 24 h. Tramadol was administered for postoperative analgesia, with no chemical effect on wound healing.

Animals were euthanized on postoperative day 1 (G1), day 2 (G2), day 3 (G3), or day 4 (G4), corresponding to the stages of the acute inflammatory response. General anesthesia was induced as described above. Blood samples were obtained immediately before euthanasia, which was performed through transection of the heart arteries and veins. Cardiac blood collection was selected because it provides increased blood volume at the time of euthanasia and yields hematological results comparable to those obtained from other sampling sites.[19–21] Because blood collection site and sampling technique may influence hematological measurements, the same sampling protocol was used for all animals throughout the study.[19–21] The blood samples were collected into ethylenediaminetetraacetic acid (EDTA) test tubes, with a minimum sample volume of 250 µL.

Each test tube was marked with the animal identification number and stored in a refrigerator at 2–8°C until delivery to the laboratory. Before shipping, the samples were placed in a Styrofoam container with thermal packs. There was no direct contact between the models and the thermal packs.

A few days before dispatching the samples, the laboratory manager issued an e-mail with a form containing details such as the scheduled shipment date, the anticipated quantity of samples and the preferred collection time. On the day of shipment, a laboratory representative sent the referral form via text message for the specific samples being dispatched. A representative of the veterinary department entered the sample information into the laboratory software (LIRIS).

Blood samples for enzyme-linked immunosorbent assay (ELISA) were collected into serum tubes provided by the laboratory. Serum concentrations were measured using a MILLIPLEX® Rat Cytokine/Chemokine Magnetic Bead Panel on a Luminex device. The test was designed for 40 samples, which were analyzed in duplicate. All samples were analyzed in a single run because each run required calibration of the MILLIPLEX® kit.

Complete blood counts were analyzed using an Advia 2120 hematology analyzer (Siemens Healthcare Diagnostics Inc., Tarrytown, USA), and differential counts were obtained automatically. The results were provided electronically in an Excel report containing data for all animals. To validate the results obtained from the hematology analyzer and manual differential counts, the director of the veterinary department or a designated deputy reviewed and verified the results using the laboratory software (LIRIS). The laboratory retained the data for 1 month before archiving it according to laboratory's standard procedures, under which data will be stored for approx. 15 years.

Statistical analysis

Categorical variables were summarized as frequencies and percentages, whereas the continuous variables were presented as ranges, means, and standard deviations. Kernel density estimation (KDE) plots were used to visualize the distribution of the data. Statistical analysis was conducted using R software (v. 4.1.1; R Foundation for Statistical Computing, Vienna, Austria) in RStudio (v. 2023.09.1). Linear mixed models with random intercepts were used. In medicine, dependent variables are often analyzed on a logarithmic scale because this transformation may improve the approximation to a normal distribution and reduce heteroscedasticity (non-constant variance across the range of the independent variable). Additionally, observations may belong to the same spatial cluster. Under these circumstances, random-intercept models provide a more appropriate analytical approach. *P*-values were adjusted for multiple comparisons using the Benjamini–Hochberg procedure to control the false discovery rate.

Results

Cytokines

TNF-α

Serum TNF-α concentrations (Fig. 1A) peaked on postoperative day 1 (9.2 pg/mL), decreased to 3.4 pg/mL on day 2, increased again to 5.3 pg/mL on day 3, and were undetectable on day 4. The difference between the maximum and minimum mean daily values was statistically significant ($p < 0.001$).

IL-18

Serum IL-18 concentrations (Fig. 1B) reached 224 pg/mL on postoperative day 1, peaked at 448 pg/mL on day 2, declined to 270 pg/mL on day 3, and remained detectable on day 4 (69 pg/mL). The difference between the maximum and minimum mean daily values was statistically significant ($p < 0.01$).

IL-6

Serum IL-6 concentrations (Fig. 1C) were undetectable on postoperative day 1, reached 54 pg/mL on day 2, peaked at 110 pg/mL on day 3, and were not detected on day 4. The difference between the maximum and minimum mean daily values was statistically significant ($p < 0.05$).

Absolute cell counts

White blood cells

The total white blood cell (WBC) count decreased significantly by 40% ($p < 0.001$), from 8,700 ±2,500 cells/μL at baseline to 5,200 ±900 cells/μL on postoperative day 1. The greatest reduction occurred on postoperative day 1, followed by partial recovery on day 2, a subsequent decline on day 3, and a further decrease on day 4 (Fig. 2A).

Lymphocytes

Absolute lymphocyte count decreased significantly by 47% ($p < 0.001$), from 7,300 ±2,500 cells/μL at baseline to 3,900 ±400 cells/μL on postoperative day 1. Lymphopenia persisted throughout the 4-day observation period (Fig. 2B).

Neutrophils

Absolute neutrophil count increased gradually by 50% during the postoperative period, rising from 1,000 ±600 cells/μL to 1,500 ±1,000 cells/μL. This increase was statistically significant ($p = 0.002$) (Fig. 2C).

Monocytes

Absolute monocyte count increased by approx. 100%, from 100 ±100 cells/μL to 200 ±100 cells/μL on postoperative day 2; however, this increase was not statistically significant ($p = 0.30$). Monocyte counts subsequently returned to baseline on the 4th postoperative day (Fig. 2D).

Eosinophils

No eosinophils were detected at baseline. Postoperatively, eosinophil counts increased significantly ($p < 0.001$) to 100 ±100 cells/μL on day 2 and reached 200 ±200 cells/μL by day 4.

Basophils

Absolute basophil counts remained very low and were barely detectable throughout the observation period.

Differential cell counts

Lymphocytes

The percentage of lymphocytes decreased by 18%, from 84 ±15% at baseline to 68.5 ±13% during the postoperative period (Fig. 2E).

Neutrophils

Differential neutrophil count increased gradually and significantly ($p < 0.001$) by 112%, from 12.2 ±6.8% on day 0 to 25.9 ±11.8% on postoperative day 4 (Fig. 2F).

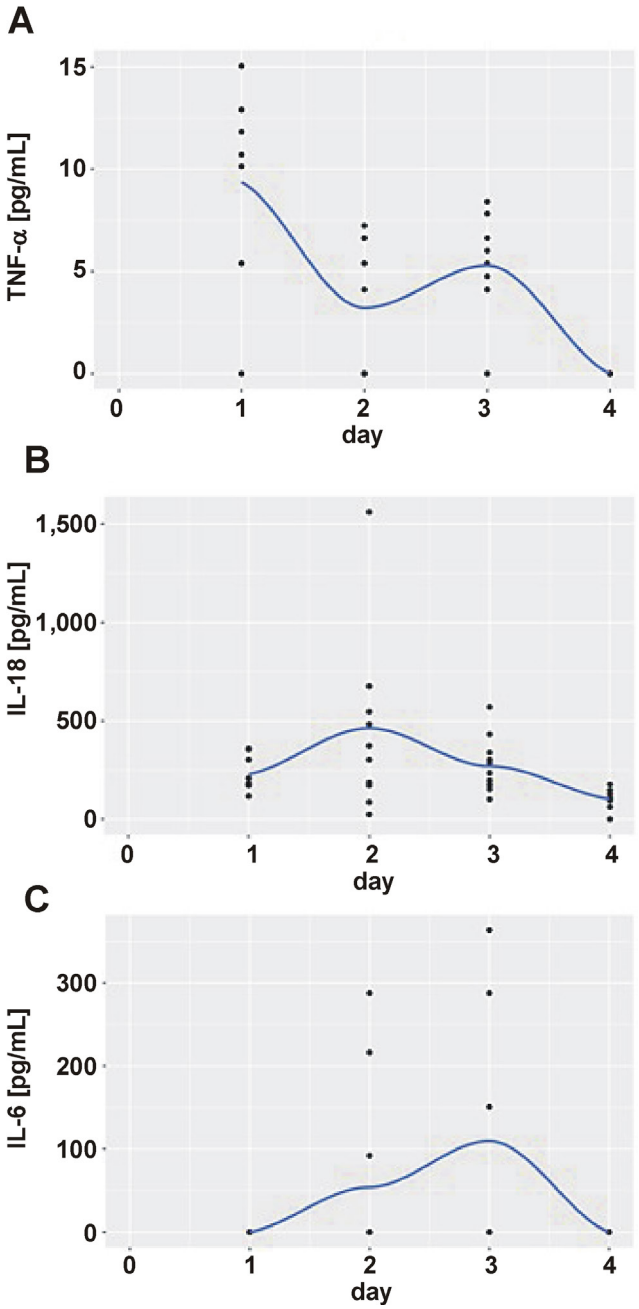


Fig. 1. Temporal changes in serum cytokine concentrations following palatal graft harvesting

A. Tumor necrosis factor (TNF)-α; B. Interleukin (IL)-18; C. IL-6.

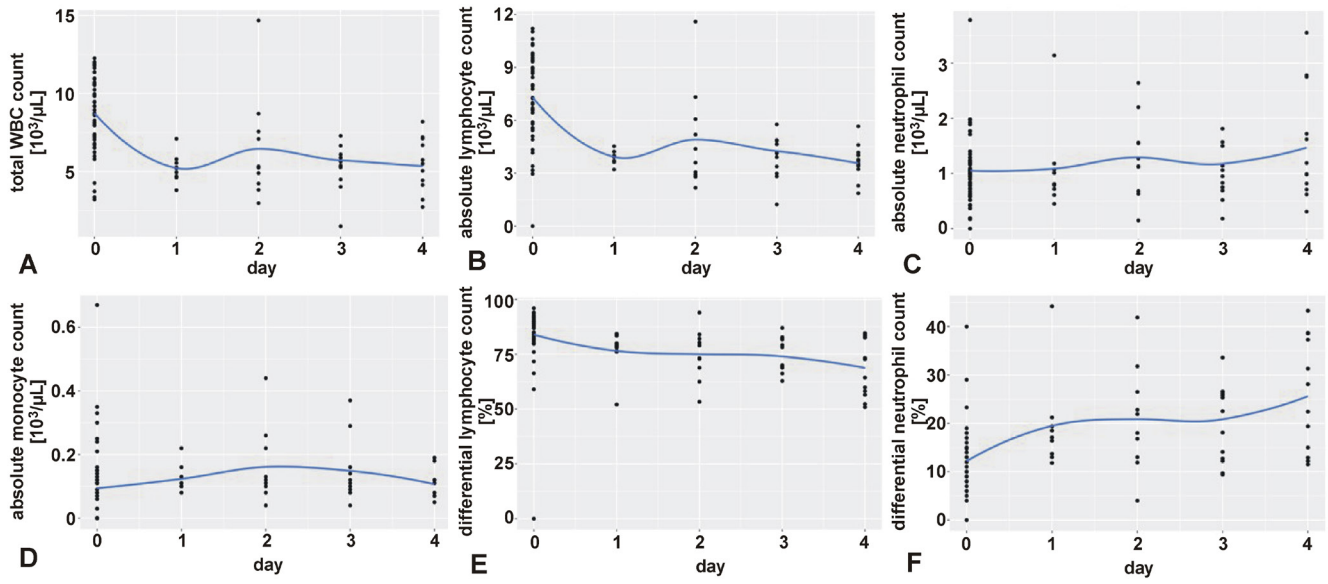


Fig. 2. Temporal changes in peripheral white blood cell (WBC) counts following palatal graft harvesting

A. Total white blood cell (WBC) count; B. Absolute lymphocyte count; C. Absolute neutrophil count; D. Absolute monocyte count; E. Differential lymphocyte count; F. Differential neutrophil count.

Monocytes

Differential monocyte count increased significantly ($p = 0.03$) by 116%, from 1.2 ±1.7% at baseline to 2.6 ±1.1% during the 3 postoperative days. On day 4, the value declined to 2.0 ±0.5%, remaining above baseline.

Eosinophils

Differential eosinophil count increased significantly ($p < 0.001$), from 0.3 ±0.6% at baseline to 2.8 ±2.6% during the postoperative period.

Basophils

Differential basophil count increased significantly ($p < 0.001$) from 0.0 ±0.1% to 0.4 ±0.2%.

Anemia-related parameters

Red blood cells

Red blood cell (RBC) count showed a transient postoperative decrease followed by recovery. A statistically significant reduction of 25% ($p < 0.001$) was observed, from 8.1 ±1.2 × 10^6 cells/μL at baseline to 6.1 ±0.9 × 10^6 cells/μL on day 2. The greatest decrease was observed on postoperative day 2, after which RBC count gradually recovered to baseline values (8.0 ±0.7 × 10^6 cells/μL) on postoperative day 4 (Fig. 3A).

Hemoglobin

Hemoglobin (Hgb) concentration also demonstrated a transient postoperative decline followed by recovery. Hemoglobin decreased significantly by 23% ($p < 0.05$), from 14.9 ±1.7 g/dL at baseline to 11.5 ±1.8 cells/μL on postoperative day 2, and returned to baseline values (15.0 ±0.9 g/dL) on the 4th day (Fig. 3B).

Hematocrit

Hematocrit (HCT) exhibited a transient postoperative decrease followed by recovery. The values decreased significantly by 23% ($p = 0.035$), from 42.8 ±5.8% at baseline to 35.8 ±4.8% on postoperative day 2, and returned to baseline by postoperative day 4 (46.2 ±2.0%) (Fig. 3C).

Mean corpuscular volume

Mean corpuscular volume (MCV) increased initially and then gradually declined. The value increased significantly by 10% ($p < 0.001$), from 54.1 ±4.8 fL at baseline to 59.3 ±4.3 fL on postoperative day 2, and remained elevated on postoperative day 4 (58.2 ±4.4 fL) (Fig. 3D).

Mean corpuscular hemoglobin

Mean corpuscular hemoglobin (MCH) showed a slight but statistically significant increase ($p < 0.001$) throughout the 4-day postoperative observation period (Fig. 3E).

Mean corpuscular hemoglobin concentration

Mean corpuscular hemoglobin concentration (MCHC) decreased slightly but significantly ($p < 0.001$) by 6%, from 34.1 ±1.6 g/dL at baseline to 32.1 ±1.9 g/dL on postoperative day 2. Subsequently, the value increased by 1 unit on postoperative days 3 and 4 (Fig. 3F).

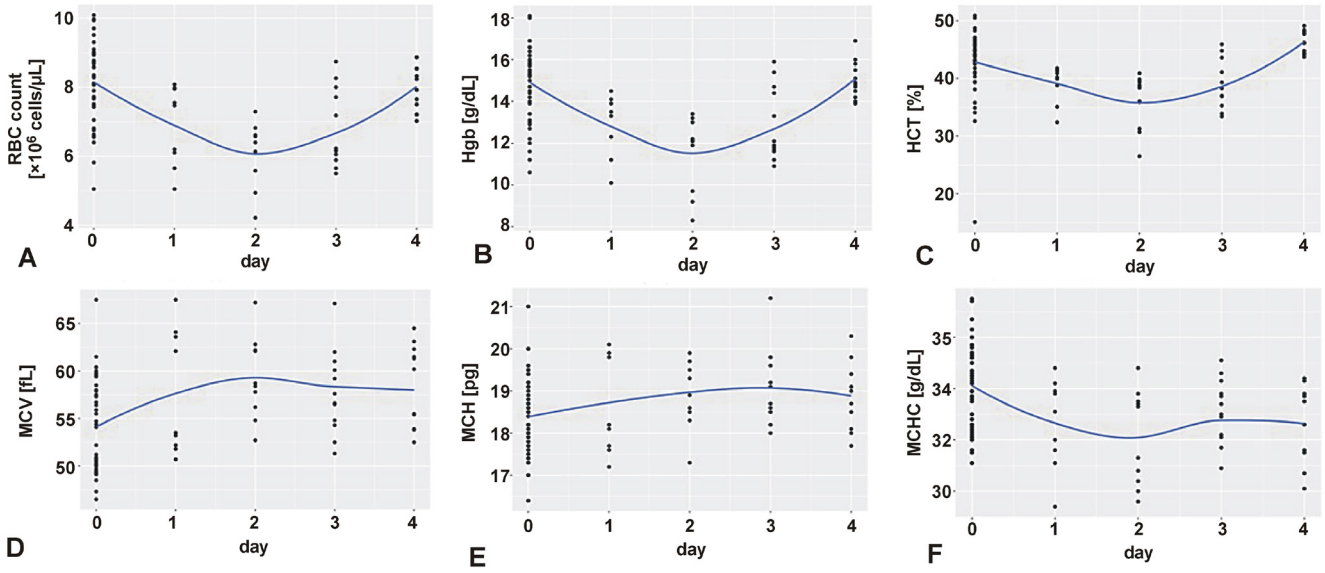


Fig. 3. Temporal changes in anemia-related hematological parameters following palatal graft harvesting

A. Red blood cell (RBC) count; B. Hemoglobin (Hgb); C. Hematocrit (HCT); D. Mean corpuscular volume (MCV); E. Mean corpuscular hemoglobin (MCH); F. Mean corpuscular hemoglobin concentration (MCHC).

Discussion

To the best of our knowledge, this study is the first to characterize the SIR following palatal graft harvesting.

Serum cytokine concentrations peaked during the acute phase: TNF-α at 12–24 h, IL-18 at 24–48 h, and IL-6 at 48–72 h. Following elective surgery, TNF-α is among the earliest and most potent mediators of the subsequent host inflammatory response. The primary sources of TNF-α synthesis are monocytes/macrophages and T cells.[22] The half-life of TNF-α is less than 20 min. Despite its brief half-life, TNF-α can induce marked metabolic and hemodynamic changes and activate mediators in the cytokine cascade.[23]

The prolonged elevation of serum TNF-α concentrations observed in the present study (3 days), despite its short half-life, may be attributed to the secondary healing characteristics of palatal graft harvesting. Exposure of the underlying bone and disruption of epithelial continuity in the middle of the palate may result in sustained cellular injury and prolonged TNF-α production.

IL-18 is a proinflammatory cytokine produced by activated macrophages. It promotes the early resolution of bacterial infections.[24] Bacterial products can stimulate IL-18 production from monocytes.[25] Previous studies have demonstrated significantly elevated circulating IL-18 concentrations during sepsis for up to 21 days, with particularly pronounced increases in gram-positive sepsis.[26] Consequently, IL-18 may serve as a systemic marker of the bacterial challenge associated with the surgical site. The high circulating levels of IL-18 observed in the present study may be attributed to the secondary healing nature of the oral wound, exposing the rats to a greater bacterial challenge.

A review demonstrated that the serum IL-6 concentrations typically peak on the first postoperative day following elective surgery.[27] In contrast, IL-6 levels in the present study peaked on postoperative day 3. This delayed peak may be attributed to the secondary healing pattern, which could sustain IL-6 production through the prolonged release of TNF-α during postoperative days 1–3. TNF-α induces IL-6 production.

The same review also demonstrated that IL-6 levels are consistently associated with the magnitude of surgical trauma.[27] Peak IL-6 concentrations following minor surgical procedures (inguinal hernia repair and cholecystectomy) were 13 pg/mL and 77 pg/mL, respectively. Corresponding peak concentrations following moderate surgical procedures (prostatectomy, total hip replacement, colorectal cancer resection, and total knee replacement) were 62 pg/mL, 140 pg/mL, 161 pg/mL, and 321 pg/mL, respectively. In contrast, major surgical procedures (abdominal aortic aneurysm repair, major liver resection, open cardiac surgery) were associated with peak IL-6 levels of 248 pg/mL, 345 pg/mL and 428 pg/mL, respectively.[27]

A difference in serum IL-6 concentrations was also observed between minimally invasive and open surgical procedures.[27] This difference was particularly evident when comparing laparoscopic and open rectopexy (21 pg/mL vs. 111 pg/mL), laparoscopic and open cholecystectomy (62 pg/mL vs. 95 pg/mL), laparoscopic and open gastrectomy (44 pg/mL vs. 129 pg/mL), laparoscopic and open abdominal hysterectomy (19 pg/mL vs. 166 pg/mL), laparoscopic and open miscellaneous colorectal resection (140 pg/mL vs. 393 pg/mL), and endovascular and open aneurysm repair (116 pg/mL vs. 332 pg/mL).

Serum IL-6 levels are associated with both the magnitude and invasiveness of the surgical procedure. In the present study, the peak serum IL-6 concentration (110 pg/mL) was comparable to those reported for moderate open surgical procedures. These findings may have implications for postoperative patient management.

Therefore, IL-6 appears to be a useful marker for assessing the magnitude of the SIR after elective surgery. Based on the previous findings, it can be hypothesized that, following oral and periodontal surgery, prolonged serum TNF-α elevation reflects persistent cellular damage (primary vs. secondary healing), IL-18 indicates the degree of bacterial challenge, and IL-6 reflects the magnitude of the surgical intervention.

Monitoring postoperative serum concentrations of TNF-α, IL-6 and IL-18 may therefore be clinically valuable. Assessment of these cytokines may be predictive of the onset of infection or the need for urgent attention to ensure optimum wound healing after oral and periodontal surgery.

The present study revealed changes in WBC counts following palatal graft harvesting. A significant postoperative reduction in total WBC count was observed, which was primarily attributable to a marked decrease in circulating lymphocytes. The linear decrease continued for the 96-h observation period. In contrast, previous studies involving cardiovascular, orthopedic, abdominal, gynecological, and urological surgeries have generally reported postoperative increases in total WBC counts.[28] One possible explanation for this discrepancy is the extent of surgical trauma. Major surgical procedures induce a pronounced cortisol-mediated stress response, resulting in increased neutrophil release from the bone marrow and marked peripheral neutrophilia, which increases the total WBC count. Oral and periodontal surgical procedures are substantially less invasive than these major operations. Consequently, the relatively limited surgical trauma associated with palatal graft harvesting may not elicit a marked increase in neutrophils. Instead, the postoperative reduction in total WBC count appears to result primarily from the marked decrease in absolute lymphocyte count.

In the previous studies, we evaluated the local time-related changes in the density of inflammatory infiltrates following palatal graft harvesting in rats.[29,30] Three weeks after the surgery, the density of inflammatory infiltrates remained high in the central part of the wound. The differences between the SIR response observed in the present study and those reported after major surgical procedures may therefore be explained by the secondary wound healing characteristic of palatal graft harvesting. In the present procedure, the time frame of the inflammatory response was extended compared to primary healing in evaluated major surgeries. The use of wound dressings may help attenuate the SIR associated with secondary wound healing.

The effects of trauma on absolute WBC counts have previously been investigated in mice.[31] The results showed that both major trauma (nephrectomy) and minor trauma (e.g., injection, bleeding), as well as surgical stress, induced a significant decrease (50–70%) in absolute lymphocyte count. The decline was gradual, with the greatest reduction occurring within the first postoperative day. These findings are consistent with those of the present study. Major trauma resulted in decreases in both B and T lymphocytes. Minor trauma affected only B lymphocytes. In addition, major trauma induced a three- to four-fold increase in absolute neutrophil counts, whereas no significant neutrophil response was observed following minor trauma. In both major and minor trauma models, hematological parameters generally returned to baseline within 24 h. Repeated exposure to trauma after recovery elicited a renewed trauma response. Bilateral adrenalectomy abolished the lymphocyte response to both major and minor trauma and reduced the neutrophil response to major trauma by more than 50%, indicating that stress hormones play an important role in mediating these changes. The authors proposed that reduced migration of lymphocytes into the circulation represented the most likely underlying mechanism.[31]

Another study evaluated the potential causes of postoperative lymphopenia by comparing the effects of major surgery, blood loss and psychological stress. Major surgery resulted in a 30–60% reduction in absolute lymphocyte counts and a 165–350% increase in absolute neutrophil counts within the 1st postoperative day. In contrast, blood loss and psychological stress (e.g., tooth extraction, blood donation, important examination) did not alter absolute lymphocyte counts during the same period. Shortly after these events, an increase in absolute neutrophil counts was observed following minor tissue trauma, such as blood donation or tooth extraction. However, no increase was detected after psychological stress alone, and the neutrophil response to minor trauma (20–55%) was substantially smaller than that observed after major surgery. It was concluded that the degree of tissue trauma is a key determinant of postoperative changes in circulating cellular immune hallmarks.[32]

The marked reduction in absolute lymphocyte counts and the moderate increase in absolute neutrophil counts observed in the present study are consistent with the response to minor surgical trauma. However, unlike previous reports in which these changes resolved within 24 h, the alterations persisted throughout the 4-day observation period, resembling continuous trauma creation. To the best of our knowledge, this is the first study to demonstrate the cellular systemic immune response following palatal graft harvesting. The observed stress response is not only psychological but also physical. Future studies should investigate the SIR associated with different soft tissue harvesting techniques and evaluate the effects of different agents that may modify this response.

The present study also revealed the postoperative changes in RBC count, HCT and Hgb levels following palatal graft harvesting. Significant postoperative reductions

in all 3 parameters were observed during the first 48 h after surgery, with complete recovery to baseline values by postoperative day 4.

Previous studies have reported a longer duration of postoperative anemia following major surgeries.[33] After open aortic surgery, the acute-phase response (APR) has been shown to induce anemia, with RBC counts returning to baseline only after 45 days, whereas Hgb concentrations remained reduced for an even longer period.[33] It may be speculated that iron deficiency is more involved in Hgb formation than in blood cell production.[34] Acute-phase response has been well documented following lower limb revascularization and aortic surgery.[35] Functional iron deficiency is considered the principal underlying mechanism and may persist for more than 6 weeks. Cytokines stimulate macrophages, blocking iron metabolism and blood cell production.[36]

Mohammed et al. reported postoperative anemia following minor surgical procedures, including herniorrhaphy and arthroscopy.[37] Anemia peaked on postoperative day 3 and returned to baseline by day 7. These findings suggest that the magnitude of anemia is related to the degree of associated stress. Factors influencing postoperative anemia include operative time, the extent of tissue injury (superficial vs. major intra-abdominal surgery), and the occurrence of postoperative complications.[37]

Compared with major surgical procedures, oral and periodontal surgery is considerably less invasive. Therefore, the shorter duration of postoperative anemia observed in the present study (approx. 2 days), its lower magnitude (25%), and the rapid recovery to baseline within 4 days may be explained by the relatively limited surgical trauma associated with palatal graft harvesting.

Serial assessment of complete blood counts and inflammatory cytokines may also be useful for evaluating the efficacy of different surgical harvesting techniques and patient-related treatment approaches. Future studies combining serial hematological and cytokine measurements with new technologies may provide additional insight into the wound healing process following palatal graft harvesting. A pilot study used digital imaging technology and three-dimensional image analysis software to evaluate healing dynamics after free palatal graft harvesting and demonstrated progressive reductions in palatal soft tissue volume and thickness.[38] Combining serial hematological and cytokine analyses with 3D digital assessment may provide a reproducible approach for investigating the cellular and humoral mechanisms underlying changes in soft tissue thickness and volume after graft harvesting. Future studies should also evaluate cytokine-modulating therapies and compare alternative graft harvesting techniques.

Limitations

To the best of our knowledge, this study is the first to characterize the SIR following palatal graft harvesting. The observed response resembles that reported after other minor surgical procedures. Nevertheless, several limitations should be acknowledged. Although the rat model provides valuable insights into postoperative inflammatory responses, inherent differences exist between rodents and humans. Only male Wistar rats were used in the present study; therefore, future investigations should include female rats to evaluate potential sex-related differences in the inflammatory response. In addition, the number of animals in each experimental group was relatively small, age-related differences were not investigated, and other potentially relevant variables, such as metabolic parameters, were not evaluated. Consequently, extrapolation of these findings from a small-animal model to humans should be undertaken with appropriate caution.

Conclusions

Based on the observed systemic inflammatory response following palatal graft harvesting, oral and periodontal surgical procedures may generally be considered minor surgical interventions. Serial assessment of complete blood counts and inflammatory cytokines may provide valuable information for postoperative monitoring and may help optimize wound healing following oral surgery.

Ethics approval and consent to participate

The study protocol was approved by the Ethics and Institutional Animal Care and Use Committee of Tel Aviv University, Israel (approval No. TAU – MD–IL-2402-109-3; February 1, 2024). All animals received humane care throughout the study. The experimental design complied with the Animal Research Reporting of In Vivo Experiments (ARRIVE) guidelines.

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

Can adhesive cementation improve the strength of glass-ceramics by filling surface defects?

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Abstract

Background. The susceptibility of glass-ceramics to surface defects can weaken their mechanical performance.

Objectives. The aim of the present study was to investigate the effect of cementation on the load-to-failure of 2 glass-ceramic materials in the presence and absence of an induced surface defect.

Material and methods. Bars of lithium disilicate – LD (Initial LiSi Block; GC Dental) and feldspathic – F (VITA Mark II; VITA Zahnfabrik H. Rauter) ceramics, as well as a dentin analog, were prepared for cementation. The ceramic specimens were assigned to 5 groups: untreated (no cementation); indented (I); cemented (C); indented and cemented with defect filling (CI); and indented and cemented without defect filling (CIN). Flexural strength was measured for the non-cemented specimens, whereas the load-to-failure was evaluated for all cemented groups, using the ball-in-hole test on bar specimens ($1 \times 1 \times 12$ mm). Finite element analysis (FEA) was performed for the groups without induced defects. Data was analyzed using the one-way analysis of variance (ANOVA) and Tukey's post hoc test ($\alpha = 0.05$).

Results. One-way ANOVA revealed significant differences among the groups for both materials ($p < 0.001$). For LD, the load-to-failure values were: LDC (16.7 ±2.9 N)^{A} > LD (14.6 ±2.6 N)^{B} = LDCI (13.9 ±3.0 N)^{B} > LDCIN (10.5 ±1.7 N)^{C} > LDI (6.9 ±1.4 N)^{D}. For F, the values were: FC (13.7 ±3.7 N)^{A} = FCIN (13.1 ±3.3 N)^{A} = FCI (11.8 ±3.3 N)^{A} > F (8.1 ±1.3 N)^{B} > FI (5.1 ±0.4 N)^{C}. Indentation significantly reduced the flexural strength of the non-cemented ceramics ($p < 0.001$). Finite element analysis showed that cementation reduced the maximum principal tensile stress by 44% for LD and by 62% for F.

Conclusions. Filling surface defects with cement increased the load-to-failure of LD, but did not significantly affect F. Overall, the mechanical performance of both glass-ceramics was influenced by the cementation process, whereas the effect of defect filling was material dependent.

Keywords: cementation, flexural strength, glass-ceramics

Cite as
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Highlights

- Cementation significantly improves the load-to-failure of both lithium disilicate (LD) and feldspathic (F) ceramics.
- The presence of a defect reduced the load-to-failure by 56% in LD and by 41% in F, highlighting the vulnerability of glass-ceramics to surface flaws.
- When defects were filled with cement, the strength of LD improved, but this effect was not observed for F.
- Cementation cannot fully compensate for strength loss due to surface defects, emphasizing the need for precise cementation protocols and defect minimization during adjustments.

Introduction

Ceramic materials, particularly monolithic restorations, were developed to address the limitations associated with porcelain-fused-to-metal (PFM) prostheses, including esthetic concerns, potential biocompatibility issues, and technical complications such as porcelain chipping.[1,2] Over the past decades, the use of dental ceramics has increased substantially owing to their favorable properties, including excellent esthetics, biocompatibility, chemical durability, low thermal conductivity, high strength, wear resistance, and translucency.[1,3] These materials are widely used for the fabrication of single crowns, veneers, inlays, onlays, and other indirect restorations because of their ability to be predictably bonded to the tooth structure and other glass-containing materials.[4,5]

Lithium disilicate (LD) is a glass-ceramic in which the base glass consists of varying SiO_2/Li_2O ratios. Most commercially available LD materials differ considerably in composition and should therefore be regarded as lithium-based glass-ceramics.[6,7] These ceramics exhibit flexural strength values ranging from 177 to 510 MPa.[8] Clinical studies have reported success rates of 88% at 5 years and 77% at 10 years for LD inlays and onlays.[9] Other long-term studies have demonstrated a survival rate of 81.9% for single crowns after 15 years[10] and 48.6% for three-unit fixed dental prostheses.[11] Fracture is the primary cause of failure in LD inlays and onlays, accounting for 61% of failures after a 10-year follow-up.[9]

Feldspathic ceramic (F) is another commonly used glass-ceramic composed primarily of feldspar, quartz, silica, and alumina as the reinforcing phase.[12] In comparison with LD, its mechanical properties are less favorable for the oral environment because of its relatively low flexural strength (60–70 MPa), which limits its clinical indications.[13] Feldspathic onlays have a 7-year survival rate of 60.5% and annual failure rates of 6.3–6.7%, with fracture accounting for 16% of failures.[14] Feldspathic veneers demonstrate a 5-year survival rate of 92% and a 10-year survival rate of 64%, with fracture (11%) and marginal defects (20%) being the most common reasons for failure.[15]

Both LD and F can be adhesively cemented using resin cement, which significantly reduces the risk of fracture and increases the failure load of ceramic restorations.[16] To enhance bond strength, various kinds of surface treatment are employed, including hydrofluoric acid etching, silica coating, and the application of ceramic primers.[3,11,17,18] Although these procedures improve adhesion, they may also introduce surface defects and microcracks that significantly reduce the flexural strength of LD.[19,20] Additional defects may be introduced during the milling process or clinical adjustments,[21,22] resulting in increased surface roughness, greater biofilm accumulation,[23,24] and reduced fatigue strength and mechanical reliability.[25] Clinical adjustments performed with diamond burs during grinding can also produce superficial surface defects. These defects may be filled by glazing the ceramic surface, thereby preserving the flexural strength of the restorative material through sealing surface flaws.[26]

The cementation of dental ceramics has been shown to increase their strength.[27] However, it remains unclear whether cementation can protect flexural strength against the incorporated defects and whether this effect depends on defect filling. Considering the adhesive interface, these findings suggest that the resin cement used during cementation may penetrate and fill surface defects, thereby protecting the restoration against failure. Lithium disilicate and F are widely used glass-ceramics that can be adhesively bonded to tooth structures. Although these materials have different mechanical properties, they share similar failure mechanisms; therefore, investigating their mechanical behavior under stress and cemented conditions may contribute to reducing clinical failure rates.

Therefore, this study investigated the effect of cementation on the load-to-failure of 2 different glass-ceramics with compromised structural integrity due to an induced defect in comparison with structurally sound specimens. The null hypothesis was that cementation, with or without defect filling, would not affect the load-to-failure of lithium disilicate and feldspathic ceramics.

Material and methods

Specimen preparation

To simulate indirect restorations, ceramic blocks of LD (Initial LiSi Block; GC Dental, Lucerne, Switzerland) and F (VITA Mark II; VITA Zahnfabrik H. Rauter, Bad Säckingen, Germany) were sectioned into bars measuring

1 × 1 × 12 mm, using a water-cooled diamond-coated saw (IsoMet 1000; Buehler, Lake Bluff, USA). The irregularly shaped bars were polished using EcoMet™ polishing sheets (Buehler) with grit sizes ranging from P320 to P2400 until the specified dimensions were achieved (±15%). The tooth substrate was simulated using dentin analog sheets (G10 plates; Carbotec GmbH & Co. KG, Aachen, Germany), which were sectioned into specimens measuring 1 × 12 mm with a standard thickness of 0.5 mm. Surface irregularities were removed following the same polishing procedure described above.

To simulate the introduction of a structural defect on the bonding surface, an indentation was created at the midpoint of the tensile surface of each ceramic bar.[26] A controlled defect was introduced in 3 of the 5 subgroups for each ceramic material. The defects were produced using a Vickers indenter (HM-124 hardness-testing machine; Mitutoyo Corp., Kanagawa, Japan), applied to the fracture surface with a load of 19.6 N for 15 s. The indentation parameters were established through pilot testing and the optimization of surface morphology through high-grit polishing. In one indented group for each material, the defect was intentionally filled with wax to prevent resin cement penetration into the defect (subgroup CIN).

The distribution of groups according to the restorative material, the presence of defect, and the cementation condition is presented in Table 1. Table 2 summarizes the materials used and their compositions.

Table 1. Specimen images and the distribution of groups according to the restorative material, the presence of defect, and the cementation condition

Ceramic	Image	Group	Defect	Cementation
LD		LD	no	no
		LDI	yes	no
		LDC	no	yes
		LDCI	yes, filled with cement	yes
		LDCIN	yes, not filled with cement	yes
F		F	no	no
		FI	yes	no
		FC	no	yes
		FCI	yes, filled with cement	yes
		FCIN	yes, not filled with cement	yes

LD – lithium disilicate ceramic; F – feldspathic ceramic.

Table 2. Materials used, brand name, manufacturer, and composition

Material and brand name	Manufacturer	Composition [wt%]
Lithium disilicate ceramic Initial LiSi Block	GC Dental	SiO_2 55–80, Li_2O 10–30, and other oxides 5–20; pigments: trace
Feldspathic ceramic VITA Mark II	VITA Zahnfabrik H. Rauter	SiO_2 56.0–64.0, Al_2O_3 20.0–23.0, Na_2O 6.0–9.0, K_2O 6.0–8.0, CaO 0.3–0.6, TiO_2 <0.1
Resin cement Panavia V5	Kuraray Noritake	Bis-GMA; TEGDMA; hydrophobic aromatic dimethacrylate; hydrophilic aliphatic dimethacrylate; initiators; accelerators; silanated barium glass filler; silanated alminum oxide filler; silanated fluoroaluminosilicate glass filler; colloidal silica; dl-camphorquinone; pigments
Dentin analog G10	Carbotec GmbH & Co. KG	fiber glass and epoxy resin
Ultradent Porcelain Etch	Ultradent Products Inc.	hydrofluoric acid (9%)
Clearfil Ceramic Primer	Kuraray Noritake	ethanol >80, TMSPMA >5, 10-MDP
Bonding system Tooth Primer	Kuraray Noritake	10-MDP; HEMA; hydrophilic aliphatic dimethacrylate; accelerators; water

Bis-GMA – bisphenol A diglycidyl methacrylate; TEGMA – triethyleneglycol dimethacrylate; BPA – bisphenol A; 10-MDP – 10-methacryloyloxydecyl dihydrogen phosphate; TMSPMA – 3-trimethoxysilylpropyl methacrylate; HEMA – 2-hydroxyethyl methacrylate.

The specimens assigned to the cemented groups received surface treatment on the bonding surface prior to cementation. The treatment consisted of etching with 9% hydrofluoric acid (Ultradent® Porcelain Etch; Ultradent Products Inc., South Jordan, USA) for 20 s for LD and 60 s for F, followed by the application of a ceramic primer (Clearfil™ Ceramic Primer; Kuraray Noritake, Tokyo, Japan) according to the manufacturer's instructions. The dentin analogs were also etched with 9% hydrofluoric acid for 60 s, followed by the application of a bonding system (Tooth Primer; Kuraray Noritake). Subsequently, cementation was performed for 6 groups (n = 90; 15 specimens per group), using a dual-curing resin cement (Panavia™ V5; Kuraray Noritake) according to the manufacturer's instructions. The 4 non-cemented groups (n = 60; 15 specimens per group) did not receive any surface treatment. The final specimens, as illustrated in Table 1, were stored in water at 37°C for 1 week prior to testing.

Load-to-failure test and flexural strength analysis

The load-to-failure test was performed using the ball-in-hole test (Fig. 1) with a universal testing machine (Instron 6022; Instron Ltd., High Wycombe, UK) on 15 specimens (n = 15) from each experimental group. The load was applied at a crosshead speed of 0.5 mm/min until failure. The load-to-failure [N] was recorded for all specimens. For the non-layered groups (LD, LDI, F, and FI), flexural strength [MPa] was calculated according to ISO 6872:2015, using the following formula:

$$\sigma = \frac{3Fl}{2bd^2} \left[\text{MPa}\right]$$

where:
σ – flexural strength [MPa];
F – maximum load applied load [N];
l – distance between the 2 points of support [mm];
b – width of the specimen [mm]; and
d – thickness of the specimen [mm].

To evaluate the maximum principal stress, a three-dimensional (3D) finite element analysis (FEA) was conducted, replicating the dimensions of the in vitro specimen while maintaining a standardized cement layer thickness of 100 µm. The geometries were created using computer-aided design (CAD) software (Rhinoceros, v. 5.0 SR8; McNeel North America, Seattle, USA) and subsequently exported in the STEP format to the analysis software (ANSYS, v. 17.2; ANSYS Inc., Canonsburg, USA). A 10% convergence criterion was applied, and the mesh was generated using tetrahedral elements. The mechanical properties of each material were defined assuming isotropic behavior. The maximum principal tensile stress at the interface was analyzed for the defect-free groups, using the elastic modulus and Poisson's ratio (ν) of the materials (LD: 95 GPa, ν = 0.25; F: 48.7 GPa, ν = 0.23),[27] under an applied load of 10 N.

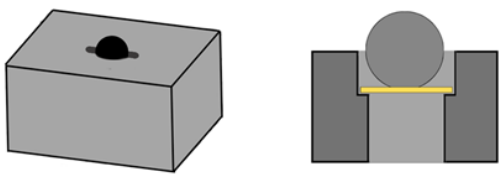


Fig. 1. Diagram and cross-section of the ball-in-hole setup

Statistical analysis

Statistical analysis of the data obtained from the three-point bending test was performed using the one-way analysis of variance (ANOVA) with a 95% confidence level, followed by Tukey's post hoc test. The load-to-failure data was analyzed to identify differences among the groups for each ceramic separately. The flexural strength values were compared between the non-cemented groups of each ceramic to validate the experimental method and support the discussion in relation to the available literature. Stress distribution was qualitatively analyzed to describe the maximum tensile strength in the defect-free groups.

Results

One-way ANOVA revealed significant differences in the load-to-failure among the groups for both LD ($p < 0.001$; $F = 34.3$) and F ($p < 0.001$; $F = 25.8$) (Table 3). As LD and F are distinct materials, this study was not designed to compare them directly. Instead, the experimental conditions were evaluated independently for each ceramic material.

The presence of a single defect reduced the load-to-failure. Representative images of the indentations are shown in Fig. 2. Based on this reference, the cemented LD group without defects (LDC) exhibited the best mechanical performance (Table 3). Therefore, the cementation procedure increased the load-to-failure values of LD under all evaluated conditions, including specimens without defects and those with defects (whether filled with cement or not). Moreover, filling the defect with cement resulted in higher load-to-failure values than leaving the defect unfilled.

For F, the presence of a defect reduced the flexural strength by 41%. Cementation improved its mechanical performance regardless of the presence of a defect, or whether the defect was filled with cement or not (Table 3).

Finite element analysis revealed that the cemented groups exhibited lower stress concentrations at the interface than the non-cemented groups (LDC: 51 MPa and FC: 35 MPa vs. LD: 91 MPa and F: 92 MPa, respectively). These findings suggest that cementation reduced the maximum principal tensile stress by 44% for LD and by 62% for F. Figure 3 presents the stress distribution color maps for the LD, LDC, F, and FC groups.

Table 3. Mean load-to-failure values for all groups and flexural strength values for the non-cemented groups, according to the restorative material, the presence of defect, and the cementation condition

Group	Load-to-failure [N]	Statistical significance (ANOVA)	Flexural strength [MPa]	Statistical significance (ANOVA)
LD	14.6 ±2.6^{B}	$p < 0.001$ $F = 34.3$	224 ±53	$p < 0.001$ $F = 71.2$
LDI	6.9 ±1.4^{D}		98 ±26	
LDC	16.7 ±2.9^{A}		–	–
LDCI	13.9 ±3.0^{B}		–	–
LDCIN	10.5 ±1.7^{C}		–	–
F	8.1 ±1.3^{B}	$p < 0.001$ $F = 25.8$	122 ±24	$p < 0.001$ $F = 35.7$
FI	5.1 ±0.4^{C}		72 ±21	
FC	13.7 ±3.7^{A}		–	–
FCI	11.8 ±3.3^{A}		–	–
FCIN	13.1 ±3.3^{A}		–	–

The same superscript capital letters represent no statistical difference between the groups, according to Tukey's post hoc test.

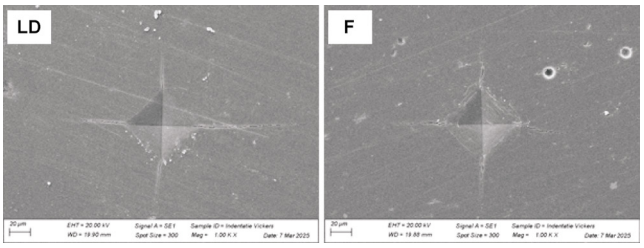


Fig. 2. Representative images of the indentations

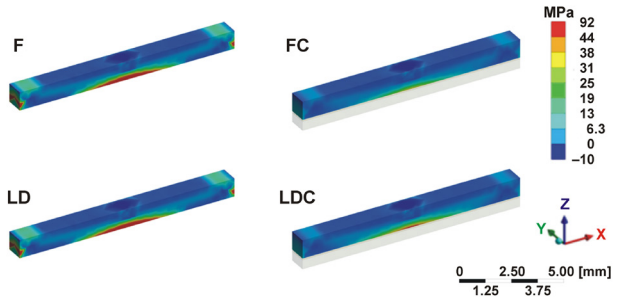


Fig. 3. Maximum principal stress (tensile stress) in the evaluated feldspathic and lithium disilicate ceramic groups – non-cemented (respectively F and LD) and cemented (respectively FC and LDC)

Discussion

This study investigated the influence of cementation on the load-to-failure of 2 types of glass-ceramics by comparing defect-free specimens with specimens containing induced structural defects. The aim was to evaluate the effect of cementation on the load-to-failure when the defect was either filled with cement or left unfilled.

The defect simulated in this study has been used in previous investigations. It was created by a Vickers indentation positioned at the center of the tensile surface of the bar. To determine whether the indentation produced a significant effect on the flexural strength of the materials, both ceramics were evaluated with and without the induced defect. The results showed that the indentation weakened the specimens and provided a reproducible defect model.[26] Consistent with the present findings and previous studies, the reduction in flexural strength demonstrates the susceptibility of glass-ceramics to structural defects.[3,8,13,26]

According to the results, the cementation procedure increased the load-to-failure of the intact ceramic bars. This improvement in mechanical strength following cementation is consistent with the current understanding of the adhesive properties of ceramic materials.[28]

Lithium disilicate with an induced defect exhibited compromised strength. However, after cementation, the reduction in the load-to-failure was partially compensated when the defect was completely filled with resin cement. In this condition, the increase in the load-to-failure can be attributed to the adhesive procedure.[29] Although cementation counteracted part of the reduction in the load-to-failure observed in the LD specimens with defects, their strength remained lower than that of the cemented specimens without defects. The presence of elongated crystals in LD contributes to the inhibition of microcrack propagation[12] and results in high fracture strength. Therefore, after cementation, the indentation remains the weakest region within the specimen structure. Although the specimen is reinforced by resin cement within the indentation, fracture initiation and propagation still occur from this location.

For F, the reduction in performance caused by the indentation was completely counteracted after cementation, regardless of whether the defect was filled with cement or not. After cementation, all groups exhibited higher load-to-failure values as compared to the non-cemented specimens, which can be attributed to the positive effect of adhesive cementation.[29] Poor mechanical properties and the presence of porosities in the microstructure of F suggest that, after cementation, the indentation is no longer the weakest region within the specimen structure.[12] Instead, fracture initiation may occur from another mechanically vulnerable area within the material. Since no difference in the load-to-failure was observed between the F specimens cemented with or without a defect, the null hypothesis can be partially accepted.

The increase in strength resulting from the cementation procedure has been demonstrated in the literature,[29] as has the influence of surface topography on the flexural strength of glass-ceramics. These findings corroborate previous studies showing that the surface microstructure plays a critical role in the fatigue strength of cemented glass-ceramics.[30] However, no previous research has investigated whether the presence of cement within a defect affects the strength of glass-ceramics.

Clinicians should recognize that glass-ceramics differ in composition and microstructure, which influence their crystal structure, fracture toughness, and mechanical behavior under stress, ultimately affecting clinical recommendations.[8] This study demonstrates that filling a defect with cement plays a critical role in increasing the fracture strength of LD, but not of F. The strength of LD specimens depends not only on the presence of cement, but also on the effective filling of surface defects. These findings highlight the importance of precise cementation protocols, as both factors influence the overall mechanical performance. Since cementation cannot fully compensate for the loss of strength caused by surface defects, clinicians should minimize their occurrence whenever possible.

Limitations

As a limitation of this study, the non-cemented groups did not receive surface treatment with hydrofluoric acid etching, whereas the cemented groups underwent hydrofluoric acid etching. This difference should be considered when interpreting the results. Flexural strength is not the only mechanical factor determining the performance of dental ceramics, as fatigue strength may also play a crucial role in their mechanical behavior. Therefore, further studies are needed to investigate the effects of these conditions on other dental ceramics, as well as to evaluate the influence of multiple defects, defect size, and different types of cements on the mechanical properties of these materials.

Conclusions

In conclusion, the presence of defects significantly weakened specimens made of both LD and F, although cementation substantially improved their strength. Feldspathic ceramic was less sensitive to defects, further highlighting the beneficial effect of cementation on its mechanical performance. In contrast, the strengthening effect of cementation was more limited for LD. Therefore, clinicians and technicians should prioritize minimizing surface defects during adjustments, as cementation alone cannot fully compensate for the reduction in strength caused by these flaws.

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

Artificial intelligence (Grammarly) was used to check the English language.

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Original paper

Strain analysis in posterior implant-supported fixed dental prostheses – cemented compared to screwed: An in vitro study

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Abstract

Background. Research on the etiology of excessive strain at the implant neck, which may be associated with technical and biological complications, is still lacking.

Objectives. This in vitro study aimed to compare strain development at the implant neck between cement-retained and screw-retained implant-supported fixed restorations, as well as among 3 prosthesis designs, using strain gauge analysis.

Material and methods. Six prosthetic configurations were fabricated and divided according to their retention mechanism: single crowns – cement-retained and screw-retained (SC-c and SC-s, respectively); splinted restorations – cement-retained and screw-retained (SP-c and SP-s, respectively); and simple three-unit bridges – cement-retained and screw-retained (SB-c and SB-s, respectively). Two strain gauges were attached to the implant necks. A vertical load of 200 N was applied simultaneously at 3 identical loading points on each restoration. Strain measurements were analyzed statistically using ANOVA and Scheffé's post hoc contrast analysis.

Results. Cement-retained restorations transferred significantly less cervical strain to the implant neck than screw-retained restorations in all 3 prosthetic configurations tested ($p < 0.001$; SC-c < SC-s; SP-c < SP-s; and SB-c < SB-s). Single crowns transferred significantly less strain to the implant neck than the other restoration designs in both cement-retained and screw-retained groups ($p < 0.001$; SC-c < SP-c and SB-c; SC-s < SP-s and SB-s).

Conclusions. The use of cement-retained implant-supported fixed prostheses results in less strain development at the implant neck when subjected to high occlusal loads as compared to screw-retained prostheses. Splinting the adjacent implant-supported restorations does not necessarily reduce strain at the implant neck.

Keywords: cement retention, screw retention, posterior partial edentulism, strain analysis, three-unit prosthesis

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Highlights

- Cement retention reduces buccolingual strain around the implant neck during loading.
- Occlusal loading of 3 single crowns resulted in less strain development around the implant neck as compared to a three-unit bridge and 3 splinted crowns.
- Applying the same occlusal load to 2 implants in a simple bridge, 3 single crowns and 3 splinted crowns did not result in excessive cervical strain in the simple bridge.

Introduction

The consistently favorable long-term survival and success rates of posterior implant-supported fixed prostheses underscore the preference for this highly reliable treatment modality, even when narrow-diameter implants are used.[1,2]

Technical complications and failures continue to drive research into their possible causes, which are generally attributed to biomechanical factors. These imply that the balance between occlusal forces and the durability of the prosthesis, its components, or its interface with the implant has been exceeded.[3–8] This phenomenon is referred to as overload.[5,8]

As occlusal forces increase toward the posterior dentition,[9] more than 70% of occlusal contacts and occlusal forces occur in these regions.[10] Possible overload in the posterior dentition may result even from physiological occlusal forces because of the lower resilience of the implant–bone interface as compared to the tooth–periodontal ligament–bone interface. Stress generated during the masticatory function with implant-supported prostheses is therefore transmitted more directly to the implant and the surrounding bone.[11] Furthermore, the markedly reduced (approx. 8-fold) tactile sensitivity associated with posterior implant-supported fixed prostheses may further contribute to this effect, since neuromuscular biofeedback is absent.[12,13] As previously reported, these 2 additive effects of occlusal loading are concentrated primarily within the cervical region of the loaded implant.[14,15]

Excessive occlusal forces that may lead to overload can occur during parafunctional activities, such as bruxism and clenching.[16] Tooth clenching commonly accompanies bruxism, which affects approx. 28–36% of the adult population.[5,17] During static clenching, the mandible is held against the maxillary dentition in the intercuspal position. In contrast, normal functions involving multiple intercuspal contacts include mastication (at the end of each chewing cycle) and deglutition. During mastication, the occlusal contact lasts for approx. 200 ms, amounting to less than 10 min per day.[18] During swallowing, the mandible is also stabilized in the intercuspal position for an average of approx. ⅔ of a second while generating forces of about 300 N.[18,19] Swallowing occurs around 590 times over a 24-hour period.[20]

During parafunctional activity, individual clenching episodes may last 20–40 s,[21] amounting to nearly 40 min over a single night's sleep and generating considerable loads through multiple simultaneous occlusal contacts.[22] Moreover, although tooth grinding predominates during sleep, clenching is more common during wakefulness, further increasing the cumulative duration of clenching.[23,24] These 3 clinical situations – mastication, swallowing and clenching – all involve multiple contacts in the intercuspal position, yet they are infrequently simulated in laboratory studies.

Prosthetic design may modify stress distribution within the prosthetic complex, as demonstrated by previous laboratory studies.[25–27] One of the key components of prosthetic design is the choice between cement and screw retention. Each retention modality has its own advantages and limitations, and the selection between the two depends largely on clinician preference. Regarding implant and restoration survival, no significant differences between the two retention methods have been reported.[28] The prevailing belief regarding the maintenance of fixed prostheses is that screw-retained restorations are primarily associated with technical complications, whereas cement-retained crowns are more prone to biological complications.[29] The limited retrievability of cement-retained restorations as compared to screw-retained restorations has been a concern; however, current evidence suggests that newer cement-retention designs offer improved retrieval techniques.[30]

Recently, screw-retained restorations have gained popularity due to their inherent advantages, including retrievability, the elimination of cementation procedures, and the widespread adoption of digital workflows that simplify impression procedures.[31,32] However, recent clinical studies have reported increased biological complications, such as crestal bone loss around these restorations, in both short- and long-term follow-up periods.[33,34] This is in addition to the previously reported higher rate of technical complications, primarily related to screw loosening and porcelain chipping.[29,35] In contrast, in vitro studies have reported different findings regarding the crestal bone region surrounding implants, with outcomes associated with parameters such as the welding technique, the framework material and the engagement modality, which may reduce stress development in the crestal bone area.[36]

Since the primary cause of technical complications is occlusal overload, direct stress and strain measurements within implants should be obtained using strain gauge analysis. This approach enables the assessment of the actual strain generated within implants and the surrounding tissues under loading conditions, without relying on intermediate or theoretical modeling.[37]

Considering the above, when exposed to comparable occlusal loads, the stress distribution patterns within implants supporting cement-retained and screw-retained posterior three-unit fixed partial dentures (FPDs) with different prosthetic configurations should be investigated.

The purpose of this study was to compare implant-supported cement-retained restorations with their screw-retained counterparts, using strain gauge analysis across 3 common partial prosthesis designs. The 1st working hypothesis was that there would be differences among the tested configurations with regard to strain registration. The 2nd hypothesis was that the retention mechanism would not influence strain development in the cervical region of the implant.

Material and methods

This in vitro strain gauge study was performed in one of the laboratories of Vishay Precision Group Corporation (Vishay Measurements Group, Raleigh, USA) to compare 3 posterior designs of cement-retained restorations with their screw-retained counterparts under simulated occlusal loading. Owing to its in vitro design, ethical approval was not required.

Test model preparation

A resin block (PLM-4B; Vishay Measurements Group, Raleigh, USA), measuring 15.8 × 35.4 × 32 mm (Cat. No. S-116H) and exhibiting an elastic modulus similar to that of human bone (2.8 GPa), was used. Three screw-shaped, SLA (sand-blasted, large-grit, acid-etched)-coated, internal-hex implants (MIS Seven; M.I.S. Implant Technology, Bar Lev Industrial Area, Israel), each measuring 3.75 mm in diameter and 13 mm in length, were inserted into the block. The implants were fabricated from Grade V titanium (Ti-6Al-4V; density: 4.4 g/cm^3; elastic modulus: 113 GPa). The implant necks protruded 2 mm above the model surface and were positioned 6 mm apart in a straight line. Open-tray impression copings (Grade V titanium: Ti-6Al-4V; density: 4.4 g/cm^3; elastic modulus: 113 GPa) were secured to the implants and splinted with acrylic resin (DuraLay; Reliance Dental Manufacturing Co., Alsip, USA) to enhance stability and impression accuracy. An implant-level impression was made using a polyether-based impression material (Impregum; 3M ESPE, Seefeld, Germany) and a custom-made acrylic resin tray, after which a working cast was poured. Three cement-retained abutments and three conical screw-retained abutments, all fabricated from Grade V titanium (Ti-6Al-4V; density: 4.4 g/cm^3; elastic modulus: 113 GPa), were connected to the implant analogs, using a controlled tightening torque of 20 N·m.

Fabrication of prostheses

Six cast base-metal fixed prostheses, including cement-retained and screw-retained designs, were fabricated from a Co-Cr-Mo alloy (Remanium GM380; Dentaurum, Ispringen, Germany; composition: Co – 64.5%; Cr – 29.0%; Mo – 4.5%; Mn, C and Si – <2%; density: 8.2 g/cm^3; elastic modulus: 220 GPa). All frameworks were fabricated to identical vertical, buccolingual and mesiodistal dimensions, using a silicone index for standardization. Investment, casting, spruing, and framework finishing procedures were standardized and performed in a single dental laboratory, which fabricated all 6 framework configurations. Framework fit was verified using a low-viscosity silicone material by a single operator (O.R.).

The prosthetic configurations were divided into 3 groups (Fig. 1):

– 3 separate cement-retained single crowns and 3 separate screw-retained single crowns (SC-c and SC-s, respectively);
– 2 three-unit FPDs with a central pontic supported by the 2 adjacent abutments, fabricated as either cement-retained or screw-retained restorations (SB-c and SB-s, respectively); and
– 3 splinted cement-retained crowns and 3 splinted screw-retained crowns (SP-c and SP-s, respectively).

After the fabrication of all 6 restorations (3 cement-retained: SC-c, SB-c and SP-c; and 3 screw-retained: SC-s, SB-s and SP-s), the corresponding abutments were connected to the implants according to the planned prosthetic configuration, using a tightening torque of 20 N·m. Ten minutes after initial tightening, all abutment screws were retightened at 20 N·m. For the SB configurations, the central abutment was removed to accommodate the pontic design.



Fig. 1. Six implant-supported fixed assemblies – 3 cement-retained on the right and 3 screw-retained on the left

Temporary cement (TempBond NE; Kerr Dental, Brea, USA) was used for all cement-retained restorations. All abutments and screw-retained restorations were installed, and all cement-retained restorations were cemented by a single investigator (G.B.I.). The cement-retained restorations were initially seated with light finger pressure, followed by the application of a pressure of 5 kg/cm^3 for 4 min, using a Lutron FG-20KG device (Lutron Electronics, Coppersburg, USA).

Strain registration

Two strain gauges (EA-06-015EH-120; Vishay Measurements Group) were attached horizontally to the neck of each implant on opposite surfaces (buccal and lingual) before abutment and crown placement. All strain gauges were bonded using a designated adhesive (M-Bond 200; Vishay Measurements Group), as shown in Fig. 2. During load application to the restorations, the changes in electrical resistance recorded by the strain gauges were used to determine the absolute strain values generated within the implant necks. This location was selected, as it has been shown to experience the highest strain concentrations during loading of implant-supported restorations.[14,15]

The strain gauge terminals were connected to a wiring adapter interface via soldered leads (134 DFV; Vishay Measurements Group). The interface was then connected to an electronic data scanner (Strain Gauge Scanner, model 5100; Instruments Division, Vishay Measurements Group), which communicated with a notebook computer through a designated interface card mounted in the docking station.

Load application

The 3 common posterior three-unit restoration designs, in both cement-retained and screw-retained configurations, were evaluated under 3 simultaneous occlusal loading points, and the resulting strain distribution within the implant necks was recorded. This experimental setup was designed to simulate oral conditions, in which full posterior intercuspation occurs during clenching, mastication and deglutition.

A custom-made loading device was fabricated to apply a vertical load of 200 N to each of the 6 restoration assemblies through 3 individual loading pins positioned in the central fossae of the restorations. The loading points were identical for all prosthetic configurations (Fig. 3).

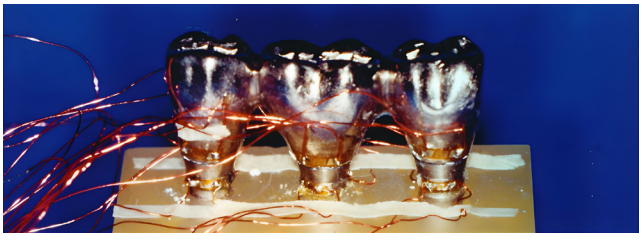


Fig. 2. Strain gauges attached to the experimental model



Fig. 3. Loading device with the test model

Each loading pin was individually adjusted using a tightening screw and 8-μm-thick articulating paper until the paper was ruptured. The loading device was then calibrated to zero, after which the load was gradually increased to 200 N. Each loading cycle lasted 20 s, followed by a 10-second unloading interval. Each prosthetic configuration was tested 3 times. After each testing session and exchange of the prosthetic configuration, the loading pins were recalibrated before the subsequent measurements.

For each loading session, strain measurements were recorded. The deformation detected by the strain gauges was expressed in microstrain (με) according to the following equation:

$$\varepsilon = \frac{\Delta L}{L} = \frac{\Delta R}{R}$$

where:

ε – strain;

ΔL – change in the strain gauge length [mm];

L – initial strain gauge length [mm];

ΔR – change in the electrical resistance of the strain gauge [Ω]; and

R – initial electrical resistance specified by the manufacturer [Ω].

Although strain measurements may represent either compression or tension, the primary outcome measure

was the absolute strain value, calculated as the sum of both components and expressed in microstrain (με) values for statistical analysis. All strain measurements were recorded by a single investigator (A.S.).

Statistical analysis

The groups were compared using one-way parametric analysis of variance (ANOVA). A p-value <0.05 was considered statistically significant. Specifically, variance analysis was performed to evaluate differences in strain distribution among the different prosthetic configurations under loading conditions. Additionally, differences among the results obtained from loading the various prosthetic configurations were assessed using Scheffé's contrast analysis. Statistical analyses were performed using IBM SPSS Statistics for Windows, v. 24 (IBM Corp., Armonk, USA).

Table 1. Strain under load at the implant neck with regard to the 2 retention mechanisms and the 3 restoration types used

Strain gauge	Restoration type	Cement retention	Screw retention	$F(p)^{\dagger}$
1	SC	162.64 ±69.41 t	886.33 ±121.68 t	400.26*
	SB	1,736.71 ±100.58 t	4,154.47 ±69.95 t	5,841.69*
	SP	1,884.40 ±59.62 t	2,130.55 ±177.08 t	26.03*
$F(p)$ contrasts‡		SP > SB > SC 2,216.43*	SB > SP > SC 2,397.84*	–
2	SC	10.27 ±1.62 t	572.66 ±49.98 c	1,897.21*
	SB	47.33 ±1.39 c	505.93 ±6.95 c	62,711.94*
	SP	48.40 ±4.86 c	384.20 ±1.93 c	61,666.52*
$F(p)$ contrasts‡		SP,SB > SC 750.42*	SC > SB > SP 161.14*	–
3	SC	48.53 ±2.66 c	9.13 ±2.47 c	1,757.70*
	SB	–	–	–
	SP	31.13 ±59.62 c	32.66 ±2.79 c	3.76
$F(p)$ contrasts‡		SP > SC 523.43*	SP > SC 596.21*	–
4	SC	529.20 ±4.21 c	946.33 ±82.35 c	383.78*
	SB	–	–	–
	SP	965.01 ±19.28 c	1,209.86 ±64.89 c	195.70*
$F(p)$ contrasts‡		SP > SC 7,321.57*	SP > SC 94.75*	–
5	SC	117.13 ±3.96 c	150.86 ±13.16 c	90.33*
	SB	349.86 ±2.06 c	374.73 ±64.42 c	2.23
	SP	233.60 ±4.01 c	105.86 ±2.74 t	10,341.01*
$F(p)$ contrasts‡		SB > SP > SC 16,890.75*	SB > SC > SP 215.53*	–
6	SC	121.00 ±7.20 c	159.53 ±16.16 c	71.13*
	SB	114.40 ±5.59 c	229.13 ±33.91 c	167.12*
	SP	186.00 ±11.77 c	334.00 ±4.69 c	2,044.37*
$F(p)$ contrasts‡		SP > SC,SB 317.64*	SP > SB > SC 242.12*	–

Data presented as mean ± standard deviation ($M \pm SD$).
SC – single crowns; SB – three-unit -bridges; SP – splinted restorations; c – compression; t – tension; † differences between cement- and screw-retained restorations; ‡ differences among the 3 types of restorations; * statistically significant ($p < 0.001$; ANOVA).

Results

Comparing retention mechanisms

The ANOVA was performed separately for each of the 3 restoration types – single crowns (SC), splinted restorations (SP) and the three-unit bridge design (SB) – to ascertain differences between the 2 retention mechanisms used (cement and screw retention). Strain gauge recordings were obtained from all 6 registration points located on the implants. The means (M), standard deviations (SD), and F-values of these recordings are presented in Table 1.

Significant differences were found between cement- and screw-retained restorations in all 3 restoration types, with cemented restorations generating less strain. In the SC configuration, all but one strain gauge exhibited less strain with cemented restorations (strain gauge no. 3). This trend was repeated in the SP assembly, where all strain gauges except for no. 5 exhibited significantly less strain with cemented restorations.

When the strain gauge recordings were combined at the implant level, as presented in Table 2, the overall strain pattern became even more apparent. Each implant exhibited greater strain under screw-retained restorations than under cemented restorations. These differences were well beyond the accepted level of significance for all implants and across all prosthetic configurations ($p < 0.001$). Therefore, the 2nd hypothesis was rejected, as substantially less strain was recorded for cemented restorations than for screw-retained restorations, regardless of the restoration design.

Table 2. Comparison of the strain values on each of the 3 implants according to the retention mechanism and the restoration type

Implant	Restoration type	Cement retention	Screw retention	$F(p)^{\dagger}$
Right	SC	86.45 ±34.81	729.49 ±52.61	1,558.19*
	SB	892.02 ±50.22	2,330.20 ±36.30	8,078.34*
	SP	966.40 ±30.09	1,257.37 ±88.50	145.32*
$F(p)$ contrasts‡		SP > SB > SC 2,309.28*	SB > SP > SC 2,511.63*	–
Middle	SC	288.86 ±3.06	477.73 ±40.82	319.28*
	SB	–	–	–
	SP	498.23 ±9.66	621.26 ±33.43	187.43*
$F(p)$ contrasts‡		SP > SC 6,391.77*	SP > SC 110.98*	–
Left	SC	119.07 ±2.87	155.20 ±9.66	192.69*
	SB	323.12 ±3.59	301.93 ±27.94	92.09*
	SP	31.13 ±59.62	32.66 ±2.79	27.79*
$F(p)$ contrasts‡		SB > SC > SP 2,244.37*	SB > SC > SP 276.92*	–

Data presented as $M \pm SD$.
† differences between cement- and screw-retained restorations; ‡ differences among the 3 types of restorations; * statistically significant ($p < 0.001$; ANOVA).

Comparing restoration types

Significant overall differences were observed among the 3 restoration types for both cement- and screw-retained designs ($p < 0.001$). The strain gauge data showed that single restorations generated the least strain as compared to the bridge and splinted crown configurations, regardless of the retention mechanism (Tables 1 and 2). Therefore, the 1st hypothesis was accepted, corroborating the findings of previous studies.[25,27]

Discussion

Parallel laboratory reports using multiple simultaneous occlusal contacts to simulate static clenching, chewing and swallowing are rare.[18,38] As noted in previous scoping reviews,[37,39] most in vitro studies evaluate simulated occlusal loading using a single loading point. Consequently, there are few comparable studies against which to evaluate our findings, apart from related publications from our own research group, owing to our unique methodological design.

The present study confirmed the 1st working hypothesis. Our findings revealed that 3 separate crowns yielded the most favorable strain distribution in both cement- and screw-retained configurations. These results are supported by previous theoretical models.[7,40] A previous finite element analysis (FEA) reached similar conclusions, demonstrating that 3 separate screw-retained crowns generated less strain than a three-unit bridge; at the level of the implant neck, 3 separate crowns produced the least strain, as did the abutment and the prosthetic screw.[41]

Disregarding the differences in our use of screw-retained prostheses and the additional restorative designs evaluated, more comprehensive reports have documented comparable findings. A recent in vitro study examined stress distribution among various cement-retained prosthetic configurations, using strain gauge analysis and the same loading protocol as the present study.[25] Regarding three-unit prostheses, the lowest strain was observed with 3 separate crowns, consistent with our findings. A simple bridge and a three-unit splint also registered significantly higher strain, as observed in the present study.[25] Similar strain gauge results for cement-retained prostheses were reported by Nissan et al. in an in vitro study, which also favored 3 separate units over a three-unit splint.[27] Another strain gauge analysis employed three-point loading on 3 screw-retained prosthetic designs.[42] Unlike the screw-retained assemblies in the present study, a simple 3-unit bridge generated the least strain at both the prosthesis level and the bone crest model as compared to a three-unit cantilever bridge. No strain gauges were placed at the implant neck, and a proximal contact point was present, both of which may have contributed to the differences between those findings and the results reported here.[42]

The 2nd working hypothesis was rejected, as the comparison of the 2 retention modes demonstrated that the cement-retained modality was significantly more favorable, with less cervical strain recorded at the implant level. Guichet et al. reported that cement-retained restorations distributed occlusal loads more evenly to the abutments because of the cushioning effect provided by the cement layer.[43] A comparative in vitro study evaluating the probability of surviving cyclic loading for cement-retained vs. screw-retained three-unit bridges found that cement-retained prostheses had a higher probability of survival than screw-retained prostheses, regardless of the implant–abutment interface.[44] Another study, using FEA, compared the biomechanical performance of the 2 retention designs.[45] The results indicated that screw-retained prostheses exhibited a higher biomechanical risk of failure at the abutment fixation screw, which was more prone to loosening or fracture.[45]

Fracture resistance has also been compared in vitro between cement- and screw-retained prostheses. Two studies demonstrated the superiority of cement retention for both five-unit and three-unit prostheses.[46,47] Gaur, comparing the strain patterns of cement-retained and screw-retained prostheses, found that under vertical loading, cement-retained prostheses generated less strain in the cervical region of the implants.[48] In that study, 2 point loads were applied to the pontics of a five-unit bridge supported by a pier abutment. Complementing our findings, the bridges were supported by 3 implants, each instrumented with 2 strain gauges placed on the mesial and distal aspects, and, as in the present study, exhibited lower overall strain with cement retention.[48]

Strain concentration within the implant neck warrants the analysis of the adjacent bone, which intimately surrounds this region. Two clinical reviews and a leading textbook in periodontology reported evidence linking cement retention with reduced crestal bone loss as compared to screw retention.[29,33,34] The structural superiority of cement-retained prostheses is well accepted.[29,42] Establishing a mechanical etiology that may contribute to peri-implant crestal bone loss is of primary importance for both understanding and managing peri-implantitis. Although this research is laboratory-based, it attempts to provide a theoretical adjunctive mechanism contributing to crestal bone loss.

An experimental setting that directly reflects strain development within the cervical region of a bone-level implant is not feasible through strain gauge analysis, as there is no space for strain gauge placement when implants are submerged within a bone substitute. The resorption of the bone surrounding implants in the crestal region is primarily considered a biofilm-mediated infectious and inflammatory process, sharing considerable similarities with the etiology and clinical characteristics of periodontitis. This premise has been described in several reviews and textbooks.[49–51] According to some authors, occlusal overload is not considered an initiating factor in peri-implantitis.[52,53]

Others have described it as an "alternative hypothesis for the cause of peri-implantitis",[50] implying a limited cause-and-effect relationship between occlusal overload and disease development. Conversely, other researchers maintain that, in addition to microbial involvement in the initiation of peri-implantitis, occlusal overload may contribute to disease progression and the exacerbation of crestal bone loss.[8,54,55] The present laboratory model may, theoretically, reveal additional mechanical factors that contribute to the initiation and possible progression of crestal bone loss in conjunction with bacterial colonization.

Limitations

Limitations of this in vitro research include the assessment of strain only along the buccolingual axis and the acquisition of data from areas in close proximity to the strain gauges, without information from more distant regions. Anteroposterior strains remain unknown, and their inclusion in the total strain dataset might influence the primary outcome parameters.

We may assume that anteroposterior strains are considerably smaller than buccolingual strains, as previously reported[56]; however, this cannot be regarded as evidence until this relationship has been thoroughly examined. The forces applied in our experiment were purely vertical and do not represent the average direction of physiological occlusal forces, which are predominantly three-dimensionally oblique.[57] Our study evaluated the various prosthetic assemblies in a "stand-alone" setting, without interproximal contacts on either side, and therefore does not fully represent clinical stress dissipation in the presence of the adjacent units. The sample size was small, and the insertion torque during the installation of abutments and screw-retained crowns remained constant, and was not compared with higher torque values exceeding 20 N·m.

For future research, a more clinically relevant setting should be considered, including the presence of neighboring teeth with interproximal contacts, thereby influencing strain magnitude through the integrity of the dental arch. Higher insertion torque values should also be examined to evaluate their impact on the same primary outcome parameters. The use of contemporary materials, such as all-ceramic prostheses and abutments fabricated from high-performance polymers, should be considered due to their reported potential influence in reducing peri-implant bone strain.[58]

Conclusions

Within the limitations of this in vitro study and based on its results, cementing posterior implant-supported prostheses may be considered as a modality for reducing strain at the implant neck in comparison with screw-retained restorations. Prosthetic design influences stress distribution within posterior three-unit implant-supported restorations, with 3 separate crowns demonstrating the lowest inherent strain.

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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Original paper

Global prevalence of orofacial pain associated with myofascial, temporomandibular joint, cranial nerve, and dentoalveolar disorders: A meta-analysis

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Abstract

Background. Orofacial pain, particularly when chronic and unrelated to dental pathology, presents a considerable diagnostic and therapeutic challenge. To address these issues, the International Classification of Orofacial Pain (ICOP), the first comprehensive international classification of orofacial pain, was published in 2020.

Objectives. The aim of this study was to determine the global prevalence of orofacial pain of different origin in accordance with the ICOP classification.

Material and methods. A systematic search of MEDLINE and Scopus was conducted for observational studies published between 2004 and 2024. Although data were initially intended to be classified according to the ICOP categories, the available studies did not permit the direct application of this classification.

Results. The pooled prevalence estimates were as follows: myofascial orofacial pain – 20.60% (95% *CI*: 9.71–38.50; 5 studies, 3,395 observations); myofascial pain combined with temporomandibular joint (TMJ) pain – 11.95% (95% *CI*: 8.85–15.96; 31 studies, 522,056 observations); TMJ pain alone – 9.51% (95% *CI*: 6.01–14.73; 13 studies, 21,407 observations); orofacial pain attributed to dentoalveolar and related structures – 27.46% (95% *CI*: 22.83–32.63; 66 studies, 548,782 observations); and orofacial pain due to cranial nerve lesions or diseases – 7.98% (95% *CI*: 3.28–18.18; 2 studies, 9,735 observations).

Conclusions. In the global population, the prevalence of myofascial orofacial pain is estimated at 21%. The prevalence of myofascial orofacial pain combined with TMJ pain is reported to be 12%. The prevalence of TMJ pain alone is estimated at 10%. The prevalence of orofacial pain attributed to the disorders of dentoalveolar and anatomically related structures is 27%. The prevalence of orofacial pain attributed to the lesions or diseases of the cranial nerves is 8%. The findings of this study should be interpreted with caution due to the substantial methodological heterogeneity observed across the included studies. This variability underscores the importance of establishing standardized criteria and reporting guidelines for orofacial pain in scientific research.

Keywords: epidemiology, myofascial pain, temporomandibular disorders, orofacial pain, cranial nerve disorders

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Highlights

- Myofascial orofacial pain affects 21% of the global population.
- Myofascial orofacial pain with temporomandibular joint (TMJ) pain is reported in 12% of individuals. The prevalence of TMJ pain alone is estimated at 10%.
- The disorders of dentoalveolar and related structures account for the highest prevalence of orofacial pain (27%).
- Orofacial pain attributed to cranial nerve lesions or diseases is the least common, reported at 8%.

Introduction

Orofacial pain, particularly when chronic and unrelated to dental pathology, represents a significant diagnostic and therapeutic challenge in clinical practice.[1–3] The literature emphasizes that orofacial pain may have a multifactorial etiology.[4,5] It is not uncommon for several pathogenic mechanisms to overlap, complicating both diagnosis and effective treatment. Due to the dense innervation and complex anatomy of the craniofacial region, pain perception in this area is characterized by high intensity and has a considerable impact on patient functioning.[4,6,7] Chronic orofacial pain is associated with a significant deterioration in quality of life (QoL), reduced occupational and social activity, and an increased risk of depressive and anxiety disorders.[8–10] Persistent pain symptoms may also lead to central sensitization, thereby perpetuating the pathological process. The diagnostic management of orofacial pain requires an interdisciplinary approach, encompassing dental, neurological and otolaryngological examinations, as well as, when indicated, psychological or psychiatric consultation. The early identification of the source of symptoms is crucial, as delayed diagnosis increases the risk of acute pain transitioning into a chronic condition.[11–13]

To address these challenges, the International Classification of Orofacial Pain (ICOP) was developed. The ICOP, published in 2020, is the first comprehensive, international classification of orofacial pain.[14] It was created through collaboration among dentists, temporomandibular disorders (TMD) specialists, neurologists, psychologists, and members of organizations such as the International Headache Society (IHS), the International Association for the Study of Pain (IASP), the American Academy of Orofacial Pain (AAOP), the International Network for Orofacial Pain and Related Disorders Methodology (INfORM), and other leading institutions.[15] The aim was to establish a common language for researchers and clinicians, enabling the precise definitions and classifications of pain disorders involving the face and the stomatognathic system.[14–16]

The ICOP employs a hierarchical structure modeled after the International Classification of Headache Disorders, 3rd edition (ICHD-3),[17] facilitating logical organization and ensuring the ease of reference. The classification identifies major categories, including: orofacial pain attributed to the disorders of dentoalveolar and anatomically related structures; myofascial orofacial pain; TMJ pain; orofacial pain attributed to the lesions or diseases of the cranial nerves; orofacial pain resembling the presentations of primary headaches; and idiopathic orofacial pain.[14] For each entity, specific diagnostic criteria are provided based on the available scientific evidence.

For example, in the context of orofacial pain, it should be noted that TMD affect up to 34% of the population.[18] The most common form of TMD for which patients seek specialist care is pain.[19–21] Projections for the coming decades suggest that the prevalence of TMD could increase from 34% to as high as 44% by 2050.[22] This underscores the public health importance of addressing orofacial pain.

Given the rising prevalence of painful conditions within the oral and facial regions, there is an urgent need for further research into the various types of orofacial pain.[14,22] Expanding the knowledge of pathophysiological mechanisms and identifying risk factors that predispose individuals to chronic pain are essential. Equally important are studies evaluating the effectiveness of diverse therapeutic strategies, including both pharmacological and non-pharmacological approaches. Advancing such research may substantially improve patients' QoL and healthcare effectiveness in this domain.

Although numerous meta-analyses and systematic reviews have examined various aspects of orofacial pain, most have concentrated on selected subtypes, such as TMD, neuropathic pain or headache-related facial pain, rather than providing a comprehensive overview based on the ICOP framework. As a result, the overall epidemiological profile of orofacial pain, organized according to the ICOP taxonomy, remains only partially characterized. Clarifying this issue may contribute to a more consistent understanding of orofacial pain patterns, and support future diagnostic and therapeutic advances.

Accordingly, the present study was designed and conducted. The aim of this study was to determine the global prevalence of orofacial pain of different origin in accordance with the classification defined in ICOP.

Methods

The present methodology was preregistered on the Open Science Framework (OSF) platform on February 19, 2025 (registration link: https://osf.io/c8x2d). Supplementary materials related to the project were also deposited in the

OSF repository (link: https://osf.io/k7eds). Registration in OSF is an accepted and recognized practice, consistent with various literature sources.[22–25]

Due to differences in subject matter and analytical methods, the collected data were divided into 2 separate publications. This division enabled a more detailed discussion of the results, ensured the consistency of interpretation, and prevented the excessive abbreviation of essential content. A comprehensive description of the database is provided in the supplementary materials.

This paper focuses on the global prevalence of disorders classified according to ICOP.[14] Data on the overall prevalence of oral and facial pain, including continental, age- and sex-specific analyses, are presented in the accompanying publication.

The systematic literature review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) 2020 guidelines,[26] using the MEDLINE and Scopus databases. The search strategy combined terms such as "orofacial pain", "facial pain" and "oral pain" with "prevalence", "epidemiology" and "population". An additional searche of gray literature was conducted on Google, supplemented by snowballing, following Wohlin's recommendations.[27]

A 20-year publication window (January 1, 2004 – December 31, 2024) was applied.[4,27] Detailed inclusion and exclusion criteria are summarized in Table 1. Only original studies published in English that confirmed the presence of pain among participants were eligible for analysis.

Study selection was performed independently by 2 reviewers (A.G. and M.W.), with disagreement resolved by the first author (G.Z.). Screening was conducted sequentially at the title, abstract and full-text levels. The PRISMA flow diagram illustrates the selection process (Fig. 1), and detailed exclusion reasons are available in the supplementary materials.

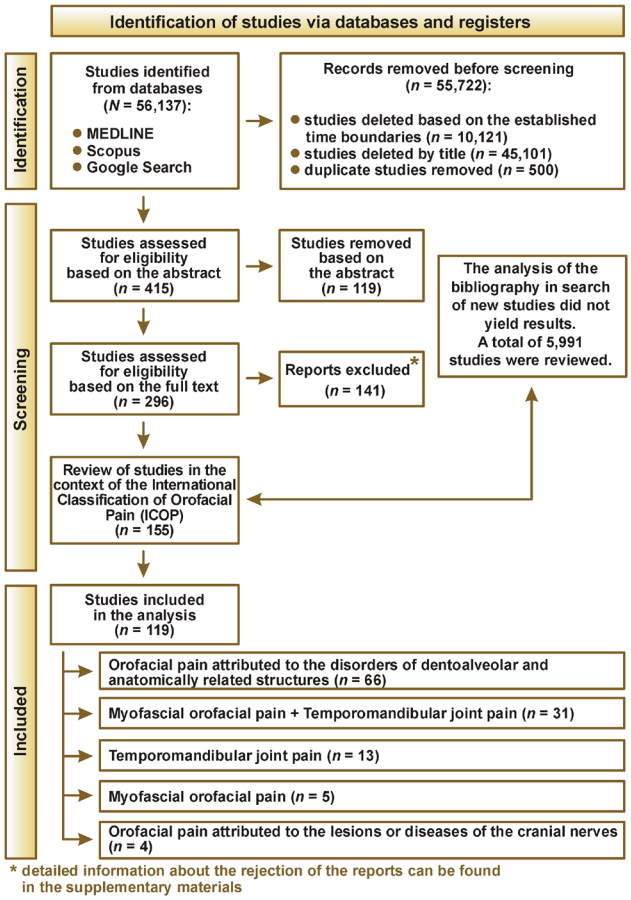


Fig. 1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flowchart of the study

Statistical analysis

The approach to statistical analysis was derived from previously published studies.[18,22,28] To investigate the prevalence of orofacial pain, we conducted a meta-analysis of proportions.[29] In this framework, each study contributed

Table 1. Inclusion and exclusion criteria

Category	Inclusion criteria	Exclusion criteria
Subject area	studies reporting on the global prevalence of disorders classified according to the International Classification of Orofacial Pain (ICOP)	studies addressing other types of pain (e.g., post-traumatic pain, neuropathic pain unrelated to ICOP)
Study population	general population samples (adults and/or children)	studies involving special or occupational groups (e.g., dentists, musicians, athletes), unless a control group was included – in which case, only the control group data were analyzed
Type of publication	original empirical research (e.g., cross-sectional, cohort studies)	narrative and systematic reviews, meta-analyses, opinion papers, case reports, conference abstracts
Language	publications in English	publications in languages other than English
Publication period	studies published between January 1, 2004, and December 31, 2024	studies published outside this time frame
Data sources	MEDLINE, Scopus, and gray literature (via Google Search)	records without full-text availability or insufficient information on study population or methods
Search terms (combinations)	"orofacial pain", "facial pain" and "oral pain" combined with "prevalence", "epidemiology" and "population" (and continent names for the gray literature search)	–
Pain confirmation	participants had to confirm the presence of pain consistent with orofacial pain criteria	studies lacking the confirmation of pain occurrence among participants
Screening process	independent screening by 2 reviewers (A.G. and M.W.); disagreement resolved by the first author (G.Z.)	–

a proportion, defined as the number of individuals reporting pain divided by the study's total sample size. To deal with the statistical challenges associated with proportions, we applied a logit transformation, which helps stabilize the variance and ensures that the pooled values, once back-transformed, remain within the [0, 1] range.[30] For the main analysis, we relied on a generalized linear mixed model (GLMM) with a logistic link.[31] This approach was chosen, as it can handle both within- and between-study variability without requiring artificial continuity corrections, making it well suited for the present dataset.[32] Between-study variance (τ^2) was estimated using maximum likelihood (ML).[33] To compute confidence intervals (*CI*) for study-specific proportions, we used the Wilson score method.[34] To check robustness, we repeated the analysis using a random-effects model based on the inverse variance method[35] with the restricted maximum likelihood (REML) estimation.[36] This alternative specification produced similar prevalence estimates and consistent measures of heterogeneity. We also conducted subgroup analyses to explore prevalence by pain type, calculating pooled proportions separately for each subgroup.[33] Heterogeneity was assessed using τ^2 and the I^2 statistic,[37] and significance was evaluated with the Wald test and the likelihood ratio test (LRT). Notably, the subgroup models incorporated group-specific variances rather than assuming a common effect.[33] To aid interpretation, subgroup estimates were back-transformed to the original proportion scale.[30] Forest plots were used to present overall prevalence, subgroup comparisons and heterogeneity statistics with 95% *CI*s. The influence of individual studies was further examined with diagnostic tools such as standardized residuals, DFFITS (difference in fits), Cook's distance, covariance ratios, leave-one-out heterogeneity checks, and DFBETAS (difference in betas).[38] Finally, we assessed publication bias using funnel plots, in which study-specific prevalence was plotted against precision.[39] Funnel plot asymmetry was evaluated both visually and statistically. In addition, we applied the regression-based method proposed by Thompson and Sharp,[40] which is particularly appropriate for meta-analyses of proportions.

Analyses were conducted using the R statistical language (v. 4.3.3; R Core Team, 2024; https://www.r-project.org) on Windows 11 Pro 64-bit (build 26100), using the packages *meta*,[41] *dmetar*,[42] *report*,[43] and *dplyr*.[44]

Results

Due to the number of analyses, their full versions together with detailed descriptions and funnel plots, the results for the influential studies are provided in the supplementary materials.

The current analysis synthesizes data from 119 observational studies published between 2004 and 2024, with excluding 2 influential studies classified as "orofacial pain attributed to the lesions or diseases of the cranial nerves".[45,46] The collected data were classified by continent and by disorder according to ICOP into the following categories:

- orofacial pain attributed to the disorders of dentoalveolar and anatomically related structures;
- myofascial orofacial pain;
- TMJ pain;
- orofacial pain attributed to the lesion or diseases of the cranial nerves;
- orofacial pains resembling the presentations of primary headaches; and
- idiopathic orofacial pain.[14]

However, after analyzing the collected publications, it was not possible to divide them according to the above-mentioned categorization. The gathered publications were divided as described in Table 2.

Pooled effect estimation of myofascial orofacial pain

A meta-analysis encompassing 5 studies, comprising 3,395 observations and 799 reported events, determined the pooled prevalence of myofascial orofacial pain to be 20.60% (95% *CI*: 9.71–38.50), as presented in the forest plot (Fig. 2). This estimate indicates a notable health burden associated with this specific type of orofacial pain across the included studies.

Substantial heterogeneity was observed across the studies, with I^2 = 99.3% (95% *CI*: 99.0–99.5) and a τ^2 value indicating considerable between-study variability, further supported by a Higgins *H* statistic of 11.79 (95% *CI*: 10.07–13.80). The statistical tests for heterogeneity confirmed this variability as highly significant, with a Wald test *Q*-value of 556.20 (df (degrees of freedom) = 4; $p < 0.001$) and LRT $p < 0.001$.

Table 2. Descriptive statistics by pain classification

Classification	Number of studies	Total number of participants	Total number of events
Orofacial pain attributed to the disorders of dentoalveolar and anatomically related structures	66	548,782	104,567
Myofascial orofacial pain combined with TMJ pain	31	522,056	29,823
TMJ pain	13	21,407	2,665
Myofascial orofacial pain	5	3,395	799
Orofacial pain attributed to the lesions or diseases of the cranial nerves	2	19,735	2,700

TMJ – temporomandibular joint.

Pooled effect estimation of myofascial orofacial pain combined with temporomandibular joint pain

A meta-analysis encompassing 31 studies, comprising 522,056 observations and 29,823 reported events, determined the pooled prevalence of myofascial orofacial pain combined with TMJ pain to be 11.95% (95% *CI*: 8.85–15.96), as presented in the forest plot (Fig. 3). This estimate highlights a significant health burden associated with this combined pain condition across the included studies.

Substantial heterogeneity was observed across the studies, with I^2 = 99.8% and a τ^2 value indicating considerable between-study variability, further supported by a Higgins *H* statistic of 21.50. The statistical tests for heterogeneity confirmed this variability as highly significant, with a Wald test *Q*-value of 13,862.48 (df = 30; $p < 0.001$) and LRT $p < 0.001$.

Pooled effect estimation of temporomandibular joint pain

A meta-analysis encompassing 13 studies, comprising 21,407 observations and 2,665 reported events, determined the pooled prevalence of TMJ pain to be 9.51% (95% *CI*: 6.01–14.73), as presented in the forest plot (Fig. 4). This estimate indicates a notable health burden associated with TMJ pain across the included studies.

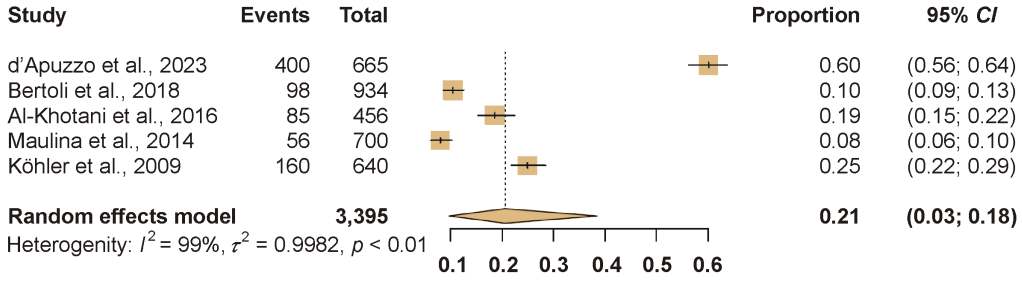


Fig. 2. Forest plot of the pooled prevalence estimates for myofascial orofacial pain (5 studies)

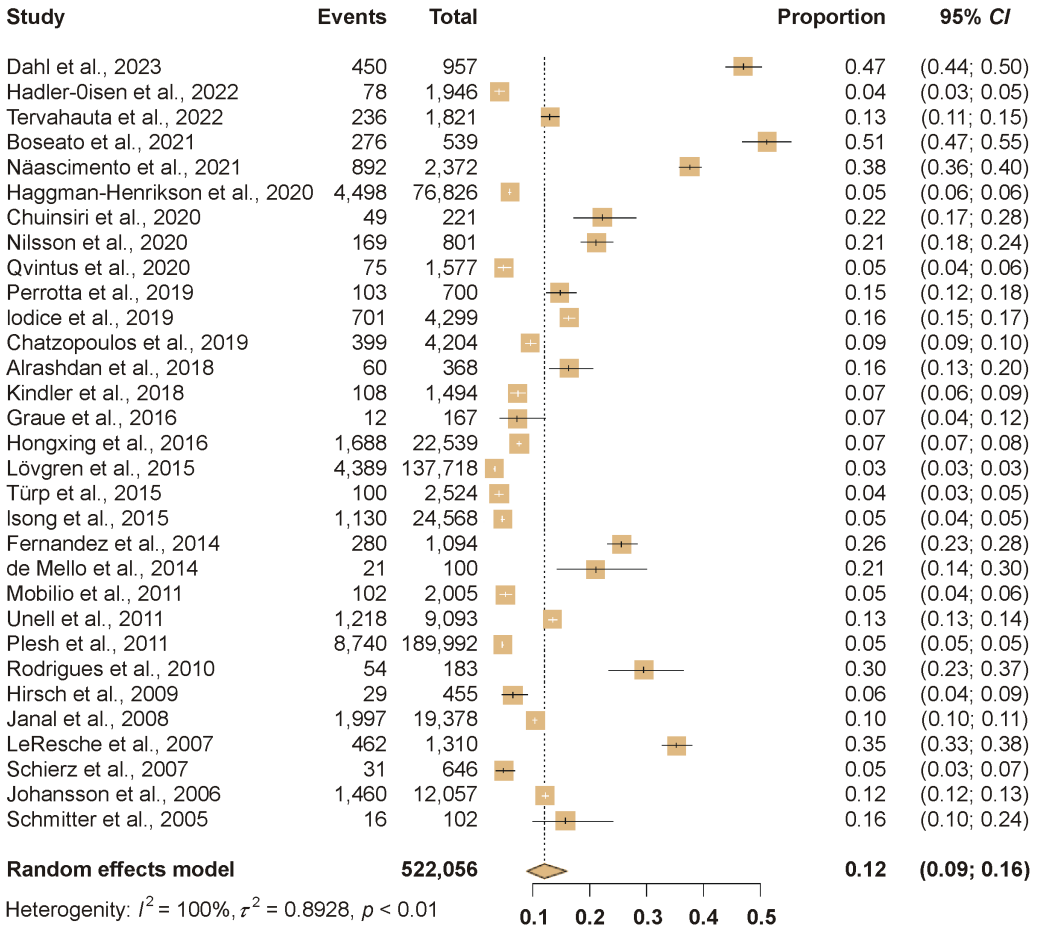


Fig. 3. Forest plot of the pooled prevalence estimates for the myofascial orofacial pain combined with temporomandibular joint (TMJ) pain (31 studies)

Substantial heterogeneity was observed across the studies, with I^2 = 99.2% (95% *CI*: 99.1–99.4) and a τ^2 value indicating considerable between-study variability, further supported by a Higgins *H* statistic of 11.49 (95% *CI*: 10.47–12.61). The statistical tests for heterogeneity confirmed this variability as highly significant, with a Wald test *Q*-value of 1,584.78 (df = 12; $p < 0.001$) and LRT $p < 0.001$.

Pooled effect estimation of orofacial pain attributed to the disorders of dentoalveolar and anatomically related structures

A meta-analysis encompassing 66 studies, comprising 548,782 observations and 104,567 reported events, determined the pooled prevalence of orofacial pain attributed to the disorders of dentoalveolar and anatomically related structures to be 27.46% (95% *CI*: 22.83–32.63), as presented in the forest plot (Fig. 5). This estimate indicates a substantial health burden associated with this specific category of orofacial pain across the included studies.

Substantial heterogeneity was observed across the studies, with I^2 = 99.8% and a τ^2 value indicating considerable between-study variability, further supported by a Higgins *H* statistic of 22.21. The statistical tests for heterogeneity confirmed this variability as highly significant, with a Wald test *Q*-value of 32,071.85 (df = 65; $p < 0.001$) and LRT $p < 0.001$.

Pooled effect estimation of orofacial pain attributed to the lesions or diseases of the cranial nerves

A meta-analysis encompassing 2 studies, comprising 19,735 observations and 2,700 reported events, determined the pooled prevalence of orofacial pain attributed to the lesions or diseases of the cranial nerves to be 7.98% (95% *CI*: 3.28–18.18), as presented in the forest plot (Fig. 6). This estimate demonstrates a notable health burden associated with this specific type of orofacial pain across the included studies.

Substantial heterogeneity was observed across the studies, with I^2 = 94.6% (95% *CI*: 83.4–98.2) and a τ^2 value indicating considerable between-study variability, further supported by a Higgins *H* statistic of 4.30 (95% *CI*: 2.45–7.55). The statistical tests for heterogeneity confirmed this variability as highly significant, with a Wald test *Q*-value of 18.53 (df = 1; $p < 0.001$) and LRT $p < 0.001$.

To investigate potential publication bias and the effects of small studies, a linear regression test was performed using the method outlined by Thompson and Sharp.[40] This procedure evaluated the extent of asymmetry in the funnel plots for the included studies. A *p*-value above 0.05 indicated that there was not enough evidence to reject the null hypothesis of symmetry, implying that the data did not exhibit notable asymmetry and, therefore, showed no signs of publication bias (Table 3).

Table 3. Results of the linear regression test for funnel plot asymmetry (Thompson and Sharp test)

Category	Mean prevalence [%]	Mean prevalence [%] ≈	*t*	df	*p*-value	Bias statistic	*SE*
Myofascial orofacial pain	20.60	21	–2.92	3	0.062	–46.55	15.94
Myofascial orofacial pain combined with TMJ pain	11.95	12	0.24	29	0.814	0.54	2.28
TMJ pain	9.51	10	–0.80	11	0.441	–4.13	5.17
Orofacial pain attributed to the disorders of dentoalveolar and anatomically related structures	27.46	27	1.68	64	0.099	6.07	3.62
Orofacial pain attributed to the lesions or diseases of the cranial nerves	7.98	8	not applied (2 studies)				

t – test statistic from the regression; df – degrees of freedom; *p*-value – probability of observing the results under the null hypothesis of no asymmetry; bias statistic – estimate of the intercept or slope indicating bias; *SE* – standard error of the bias statistic.

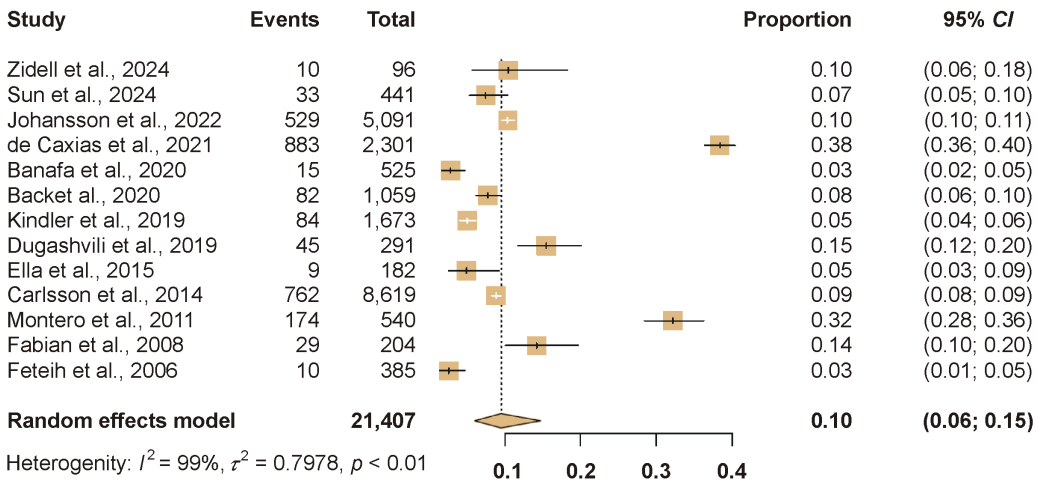


Fig. 4. Forest plot of the pooled prevalence estimates for temporomandibular joint (TMJ) pain (13 studies)

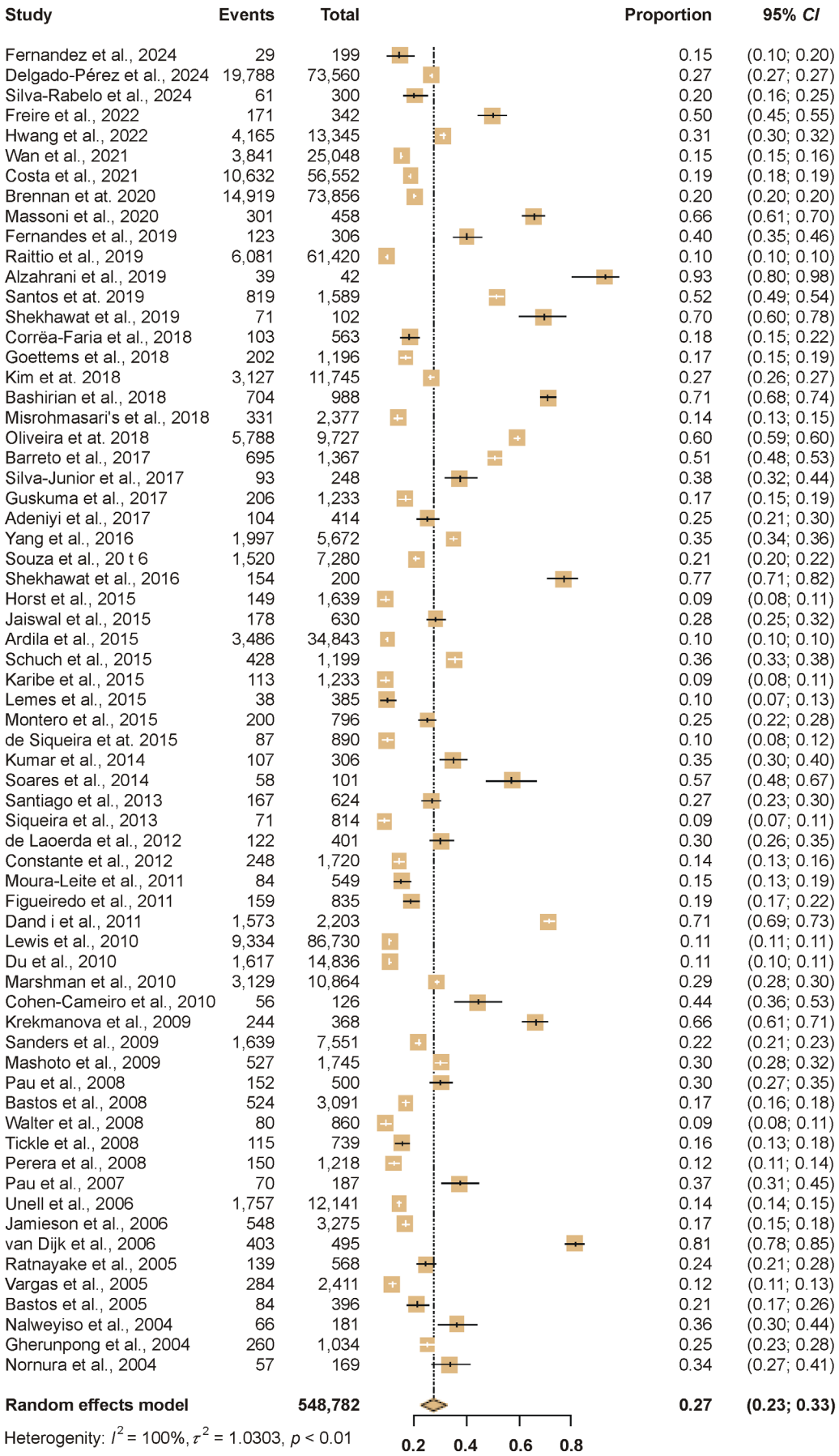


Fig. 5. Forest plot of the pooled prevalence estimates for orofacial pain attributed to the disorders of dentoalveolar and anatomically related structures (66 studies)

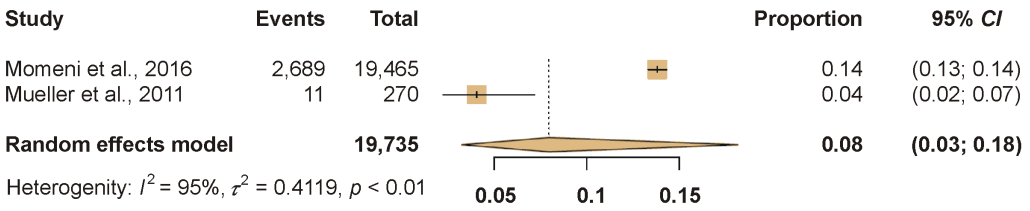


Fig. 6. Forest plot of the pooled prevalence estimates for orofacial pain attributed to the lesions or diseases of the cranial nerves (2 studies)

Discussion

The aim of this study was to determine the global prevalence of pain according to the classification set forth in the ICOP.

The first set of results concerns myofascial pain in the orofacial region, with a prevalence estimate of 20.60%. Despite the relatively small number of studies (n = 5), this proportion suggests a considerable burden of this condition. The wide *CI* reflects high heterogeneity. Previous estimates suggest myofascial pain affects 30–93% of patients, which aligns with our findings.[47] In light of these findings, the estimate obtained in the present analysis – focused on the orofacial region as a particular anatomical area – falls within the expected range and aligns with prior knowledge. Differences between these results and earlier, more general prevalence estimates should be considered acceptable, as they may reflect both the unique characteristics of the anatomical region under investigation and methodological variability across studies.

The next category concerns myofascial orofacial pain combined with TMJ pain. The prevalence of 11.95% appears lower than that of isolated myofascial pain; however, it should be noted that these data are derived from a substantially larger evidence base (n = 31, >500,000 observations). The large sample size improves reliability despite high heterogeneity.

Another category, TMJ pain alone, was estimated at 9.51%. This is a moderate value, but it pertains to a specific pain subtype that often coexists with other orofacial pain conditions. As in other categories, the results are affected by very high heterogeneity, which limits the ability to draw definitive conclusions about population-level burden, but nonetheless provides a broad outline of the global epidemiological situation.[18,22]

The epidemiology of myofascial orofacial pain combined with TMJ pain and TMJ pain alone indicates that both conditions occur in fewer than 12% of the global population. Considering the most recent meta-analysis from 2024 on TMD prevalence, estimates suggest a rate of 34%, though without differentiation between painful and non-painful forms.[18] LeResche noted in her review that the painful form of TMD affected 10% of adults over the age of 18.[48] Similarly, Yap et al., in their meta-analysis, reported that TMD pain occured in 8% of the global population,[49] which corroborates the findings of the present study.

Even higher prevalence values are observed for orofacial pain attributed to the disorders of dentoalveolar and anatomically related structures, estimated at 27.46%. This finding underscores the dominant role of dental pathologies in the etiology of facial pain. The large number of studies (n = 66) and over half a million observations make this estimate appear robust at the population level. Orofacial pain attributed to the disorders of dentoalveolar and related structures represents the most prevalent category in this study, given its direct association with highly common conditions such as dental caries, pulpitis and periodontal disease.[50–52] This type of pain is the leading cause of dental visits, as it serves as a clear warning signal of tissue damage.[1,53] Moreover, the high density of anatomical structures within the craniofacial region often results in pain radiation, which further increases the number of reported complaints.[1,53,54] Unlike rarer causes, such as neuropathic or functional pain, odontogenic pain is more readily recognized and more likely to prompt patients to seek professional care.[55–57]

Also of interest are the data on orofacial pain attributed to the lesions or diseases of the cranial nerves. In the pooled analysis, the prevalence of these conditions was estimated to be 7.98%. While noteworthy, this result should be interpreted with caution due to the very limited number of studies (n = 2) and the wide *CI*. It is useful to compare these findings with population-based studies on cranial neuralgias. Haller et al. reported that the incidence of trigeminal neuralgia (cranial nerve V) ranges from 4 to 20 cases per 100,000 per year, corresponding to 0.004–0.020% annually.[58] Schwaiger et al. found cranial neuralgias in 1.6% of individuals aged 55–94 years, suggesting a higher prevalence among older populations.[59] Wettervik et al. estimated the annual incidence of trigeminal neuralgia at 5.5 per 100,000 person-years, corresponding to 0.0055% annually.[60] Taken together, these findings indicate that the high prevalence of cranial nerve-related pain observed in the meta-analysis is not fully consistent with epidemiological data on trigeminal neuralgia. Such discrepancies may arise from differences in the definition of study entities (broader neuropathic pain vs. narrowly defined trigeminal neuralgia), study methodologies and population characteristics. This warrants further research.

The most consistent finding across all categories is the extremely high level of heterogeneity, which poses both methodological and practical challenges. This highlights

significant differences between studies in design, diagnostic criteria, population characteristics, and potential cultural or regional influences, as demonstrated in other analyses.[18,28,61] Uniform ICOP standards introduced in 2020 may improve future consistency.[14] For TMD, the diagnostic criteria (DC/TMD) were developed as early as in 2014,[62] and have since been translated and validated in multiple languages.[63] The consistent application of standardized frameworks is essential for reliable meta-analyses. The present review includes studies conducted between 2004 and 2024, meaning that most of the earlier analyses preceded the introduction of current diagnostic criteria, which may further explain the observed heterogeneity.

Heterogeneity arises from differences in diagnostic criteria, population characteristics, and study methodologies.[64,65] The ICOP standardization improves interdisciplinary communication and diagnostic accuracy. Future research should address regional data gaps, explore the determinants of pain heterogeneity, and assess the clinical value of ICOP-based diagnostic pathway.[56,57,64,66]

Conclusions

In the global population, the prevalence of myofascial orofacial pain is estimated at 21%.

The prevalence of myofascial orofacial pain combined with TMJ pain is reported to be 12%.

The prevalence of TMJ pain alone is estimated at 10%.

The prevalence of orofacial pain attributed to the disorders of dentoalveolar and anatomically related structures is 27%.

The prevalence of orofacial pain attributed to the lesions or diseases of the cranial nerves is 8%.

The findings of this study should be interpreted with caution due to the substantial methodological heterogeneity observed across the included studies. This variability underscores the importance of establishing standardized criteria and reporting guidelines for orofacial pain in scientific research.

Ethics approval and consent to participate

Not applicable.

Data availability

The data related to this article, including additional materials, are available in the Open Science Framework database at the following link: https://osf.io/k7eds/files. The script used in the analysis is available from the corresponding author upon reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Review

Physical therapy interventions for awake and sleep bruxism: What has been the approach over the last decades? A systematic review of the evidence and research gaps

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Abstract

The objective of this systematic review was to summarize the physiotherapy (PT) treatments used over the last few decades to manage awake bruxism (AB), sleep bruxism (SB) or unspecified bruxism in adults. Searches were conducted in 5 databases, and all stages of the review were performed independently by 2 reviewers. A total of 1,745 records were identified through the database searches. Of these, 86 studies underwent full-text assessment, and 13 met the inclusion criteria. An additional manual search identified 2 further eligible studies, resulting in a total of 15 studies included in this systematic review. A variety of PT modalities were reported in the literature. Manual therapy (MT) combined with occlusal splint therapy or kinesiotaping demonstrated positive effects on clinical outcomes, including orofacial pain, sleep quality, the number of trigger points, and jaw disability in individuals with bruxism. Overall, PT may be beneficial for individuals with bruxism, particularly those with associated conditions such as temporomandibular disorders (TMD) or headaches. However, the available evidence is limited and heterogeneous, and most included studies presented a high risk of bias.

Keywords: physical therapy modalities, bruxism, systematic review, evidence gaps

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Highlights

- Physiotherapy has been increasingly used to manage the adverse consequences of bruxism, although supporting evidence remains limited and of low quality.
- Muscle stretching, massage therapy and adjunctive interventions, including occlusal splints, show potential for alleviating the adverse consequences of bruxism.
- Future research should adopt the latest bruxism sub-classification.
- A biopsychosocial approach integrating patient education, motor control exercises and behavioral interventions may improve the therapeutic and rehabilitative management of bruxism.

Introduction

Musculoskeletal physiotherapy focuses on dysfunctions involving muscles, joints, bones, nerves, tendons, and related structures, with the primary aims of alleviating pain and improving function. It encompasses techniques such as manual therapy (MT), exercise, electrotherapy, relaxation techniques, movement therapy, kinesiotherapy, and patient education to address pain, fatigue and mobility limitations according to individual clinical needs.[1–7] One area of physiotherapy (PT) addresses dysfunctions affecting the head, face and neck, including the stomatognathic system,[2–4,7] with a particular emphasis on temporomandibular disorders (TMD).[8] Within this field, bruxism, a masticatory muscle behavior that may or may not be associated with TMD,[8–11] has gained increasing attention from physiotherapists.[2] International consensus statements defining the characteristics of this behavior were published in 2013 and 2018, and were subsequently updated in 2025.[9,12–14]

According to the international consensus, bruxism is not classified as a disorder but may be considered a risk factor when associated with adverse health outcomes, such as pain, severe mechanical tooth wear, and tooth damage.[12] Bruxism is categorized into awake bruxism (AB), characterized by repetitive or sustained masticatory muscle activity involving tooth contact and/or jaw bracing or thrusting during wakefulness, and sleep bruxism (SB), characterized by rhythmic or non-rhythmic masticatory muscle activity during sleep.[12] Bruxism can be assessed using self-report, clinical examination, and instrumental methods such as electromyography (EMG) and polysomnography.[15,16] Each assessment method captures a different aspect of bruxism.[12] Self-report reflects an individual's awareness of the behavior, whereas clinical examination may identify potential signs associated with bruxism, such as tooth wear, rather than the behavior itself. Instrumental assessments provide objective measures of masticatory muscle activity.[12,15]

The prevalence of bruxism in the general population is increasing. A review published in 2024 estimated a pooled prevalence of 22%, with rates of 23% for AB and 21% for SB. Both types were reported more frequently in women than in men.[17]

There is also evidence that psychosocial factors are associated with bruxism, particularly AB.[18–20] Studies suggest that stress, anxiety, depression, and certain personality traits contribute to the development of AB. For example, Manfredini and Lobbezoo reported that AB is strongly associated with psychosocial factors and psychopathological symptoms, whereas SB does not demonstrate the same pattern of associations.[18] Anxiety, stress sensitivity, depression, and specific personality traits have consistently been linked to AB, while the evidence relating psychosocial factors to SB remains less consistent.[18,21] However, the etiology of bruxism is multifactorial and may differ between SB and AB, with lifestyle factors playing an important role in both.[22–24] Furthermore, bruxism and its adverse consequences have been shown to negatively affect quality of life, jaw function, motor activity, pain, and oral health.[2,24]

When bruxism is associated with adverse outcomes, interventions are often required to manage the behavior and mitigate its consequences, such as pain and tooth wear.[2] An important aspect of its clinical presentation is its possible association with TMD.[10] Although this relationship remains controversial, several studies have investigated the association between bruxism and TMD.[10,11] Emodi-Perlman and Eli, for example, discussed the prevalence of AB and SB and suggested that excessive bruxing activity may contribute to dysfunction of the stomatognathic system, orofacial pain, and TMD.[25] A recent meta-regression analysis by Zieliński et al. estimated the global co-occurrence of bruxism and TMD to be 17%, with marked geographical variation. The authors also found that each 1% increase in the proportion of female participants was associated with a 4.4% increase in the probability of co-occurring TMD and bruxism.[11] These findings highlight the frequent coexistence of both conditions and the influence of geographical and demographic factors on this association.[11]

Occlusal splint therapy remains the most common intervention for individuals with bruxism, particularly SB, and is often combined with pharmacotherapy under the supervision of a dentist.[4,26] These approaches aim to protect the dentition, reduce masticatory muscle tension, improve sleep quality, and alleviate orofacial pain and headaches, when present.[26] Physiotherapy has not yet

been established as a standard approach for managing the adverse consequences of bruxism. However, several PT interventions may offer clinical benefits, particularly for individuals presenting with pain, jaw dysfunction and increased muscle tension.[2,27]

Numerous systematic reviews[2,26,27] have evaluated interventions for individuals with bruxism presenting with symptoms such as pain, jaw disability and muscle tension. One review investigated the effectiveness of PT for both AB and SB and examined 7 treatments, including electrotherapy and cognitive behavioral therapy, which were the most frequently studied and showed the most favorable results for reducing muscle activity and pain.[2] Another systematic review focused solely on the management of SB and its associated symptoms through pharmacotherapy, oral appliances, biofeedback, and cognitive behavioral therapy.[4] However, these reviews[2,4] were published more than 5 years ago. Since then, the concept of bruxism has evolved considerably, with important updates to its definition and assessment. Bruxism is now understood as a motor behavior of the masticatory muscles rather than a disorder, supporting an interdisciplinary management approach that includes professionals with expertise in human movement, such as physiotherapists.[2,3] Consequently, earlier reviews may not have captured important PT-related evidence. Furthermore, the review focused on SB restricted its literature search to studies published between 2007 and 2015, potentially limiting the identification of relevant evidence.[4] Although a variety of PT strategies were reported, relatively few studies were included, possibly because of the limited search strategy.[2]

A review published by our group in 2023 examined biofeedback as a primary intervention for individuals with AB.[27] The review demonstrated positive effects of auditory and visual biofeedback in reducing non-functional masticatory muscle activity and the frequency of bruxism-related muscle events. However, it also identified important limitations, including methodological heterogeneity and an exclusive focus on masticatory muscle activity without evaluating other clinically relevant outcomes that could be significant for those experiencing negative consequences of bruxism.[27]

As research in this field has expanded in recent years, several new randomized controlled trials (RCTs) and clinical trials (CTs) have investigated PT interventions for individuals with AB and SB.[28–32] Consequently, existing systematic reviews are now outdated.[2,4,26,27] There is therefore a need to synthesize the most recent evidence regarding the effectiveness of PT interventions for managing bruxism and its associated adverse outcomes. In addition, it is important to determine whether these interventions are consistent with the current understanding of bruxism.

This systematic review aims to critically evaluate and synthesize the current evidence regarding PT interventions for managing bruxism and its adverse consequences, when present, including masticatory muscle activity, muscle tension, jaw pain, functional impairment, muscle tenderness, and compromised oral health-related quality of life. Furthermore, it aims to assess the methodological quality and risk of bias of the included studies and to formulate clear directions for future research in the field of bruxism and PT.

The following questions were addressed in this review: What PT interventions have been used to manage AB and/or SB, either alone or in combination with associated conditions, and how effective are these interventions compared with other treatment approaches? Specifically, how do these interventions affect muscle activity (measured by EMG or other tools), reduce the adverse consequences of bruxism, including pain, muscle tenderness and jaw disability, and improve the quality of life of individuals with bruxism?

Material and methods

This systematic review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) statement.[33]

The review was registered in PROSPERO under registration No. CRD42021251182. All stages of the review were conducted independently by at least 2 trained reviewers. All extracted data are available from the corresponding author upon reasonable request.

Search strategy

The search strategy was developed by 2 investigators (AISOS and SAO) using the Population/Intervention/Comparison/Outcome (PICO)[34] framework under the supervision of an experienced Health Sciences Librarian. The final search strategy was based on a list of keywords derived from Medical Subject Headings (MeSH) and related to the concepts of bruxism and PT. The search was restricted to RCTs and CTs. No restrictions were applied regarding publication date, language or publication status. The electronic search was last updated in July 2023, and a manual search was conducted through November 2024 in the following databases: MEDLINE (Ovid MEDLINE® All), Embase (Ovid interface), CINAHL Plus (EBSCOhost interface), Cochrane Library Trials (Wiley interface), Web of Science (Science Citation Index Expanded (SCIE), Social Sciences Citation Index (SSCI), Arts & Humanities Citation Index (AHCI), Emerging Sources Citation Index (ESCI)). The details of the search strategy can be found in Appendix 1 (available on request from the corresponding author). Manual searches, including grey literature, were performed by screening the reference lists of the included papers using the Scopus database. In addition, members of the review team, who are experts in the field, were consulted to identify any ongoing trials on this topic.

Eligibility criteria

The inclusion and exclusion criteria were defined according to the PICO framework.

Population

This systematic review included studies involving adults (>18 years) with AB and/or SB. Bruxism could have been diagnosed by a dentist using standardized assessment methods, such as EMG, polysomnography, the oral health checklist, or other validated instruments, or identified through participants' self-report (Appendix 2). The classification of bruxism followed the international consensus on the assessment of bruxism.[10] Studies were excluded if they focused solely on children or adolescents, lacked clear information regarding bruxism classification, focused exclusively on dental conditions (e.g., missing teeth, toothache) unrelated to bruxism, or included participants with specific comorbidities, such as systemic diseases, rheumatoid conditions, arthralgia, cancer, neuropathic pain, or previous temporomandibular joint (TMJ) or neck surgery.

Studies were grouped according to the type of bruxism reported, as follows:

- AB and SB – studies that included participants with both AB and SB without reporting separate analyses;
- AB – studies including only participants with AB;
- SB – studies including only participants with SB;
- unspecified bruxism – studies that did not specify whether participants had AB or SB.

Whenever possible, information regarding whether bruxism was associated with other conditions was extracted (Appendix 2). When studies reported only the presence of bruxism without describing associated symptoms (e.g., pain), this limitations was highlighted.

Interventions of interest

Studies evaluating any form of PT that could potentially reduce the adverse consequences of bruxism, including pain, muscle tenderness and jaw disability, were eligible. Interventions of interest included MT, exercise therapy and PT techniques aimed at promoting muscle relaxation, such as stretching, massage, myofascial release, and progressive muscle relaxation. In addition, interventions commonly used within PT practice, including acupuncture, dry needling, massage therapy, electrotherapy (e.g., transcutaneous electrical nerve stimulation (TENS) and microcurrent electrical neuromuscular stimulation (MENS)), trigger point therapy, and general PT (i.e., combinations of different PT approaches), were considered eligible (Appendix 2).

Studies evaluating PT combined with other interventions were included only when the specific effects of the PT component could be identified and analyzed separately. Studies in which the effect of PT could not be isolated were excluded. Interventions based primarily on psychological approaches, such as behavioral or relaxation therapies, were also excluded.

Comparison

Eligible comparator interventions included placebo, no treatment, pharmacological therapy, behavioral interventions, occlusal splint therapy, and any other therapeutic approach.

Outcomes

All reported outcomes were considered (Appendix 2). The primary outcome was masticatory muscle activity (i.e., bruxism behavior), including clenching, grinding, jaw thrusting, and jaw bracing. Secondary outcomes included pain intensity, muscle tenderness, jaw function, jaw activity, mastication, quality of life, and patient-reported outcomes. Any additional outcomes evaluated in the included studies were also extracted.

Study design

This systematic review included RCTs and CTs, as these designs provide the highest level of evidence for evaluating treatment effectiveness. All other types of studies, such as cross-sectional studies, cohort studies, case reports, narrative reviews, systematic reviews, meta-analyses, commentaries, letters to the editor, conference papers, book chapters, protocol registrations, abstracts without full text, and animal studies, were excluded.

Time points

All assessment time points reported in the included studies were considered, including baseline, immediate post-intervention, short-term, medium-term, and long-term follow-up.

Data screening

Study selection

Search results were imported into EndNote™ (https://endnote.com) and subsequently uploaded to the Covidence platform (https://www.covidence.org), which was used to manage the screening process. The PRISMA flowchart was used to document the numbers of identified, duplicated, selected, and excluded studies.[35]

Two independent reviewers screened the titles and abstracts according to the predefined eligibility criteria. Full texts were obtained for all potentially eligible studies and for studies with insufficient information in the title or abstract. The reviewers then independently assessed the full-text articles while remaining blinded to each other's

decisions. Disagreements were resolved through consensus. If consensus could not be reached, the third reviewer made the final decision.

Data extraction

The data was extracted and organized using a pilot-tested Microsoft Excel form developed specifically for this review. The extraction form was created following a standardized process and was refined through iterative discussions among the reviewers. One reviewer independently verified all extracted information and organized the data into tables for further analysis. A second reviewer double-checked all extracted data. Any disagreements were resolved through discussion and consensus, with arbitration by a third reviewer when necessary.

Extracted information included, but was not limited to, article characteristics, participant characteristics, study design, intervention characteristics, outcomes, summary of results, data analysis, conclusions, study limitations/comments, and recommendations. When quantitative data was missing, the authors were contacted to request the unavailable information.

Risk of bias assessment

Assessment of the risk of bias (RoB) was conducted concurrently with data extraction. Two independent reviewers evaluated the quality of the included studies using a compiled set of items (CSoI) and the revised Cochrane risk-of-bias tool (RoB 2).[36,37] The RoB 2 evaluates 5 domains: the randomization process; deviations from the intended interventions; missing outcome data; measurement of the outcome; and selection of the reported results.[36,38] Each domain was rated as presenting low RoB, unclear (some concerns) RoB, or high RoB. An overall risk-of-bias judgment was assigned to each study according to standard guidelines.[36,39–41] Any disagreements were resolved by consensus.

Strategy for data synthesis

The data was summarized narratively, resulting in a descriptive analysis of the included studies. Evidence tables were prepared to summarize study characteristics, interventions, outcomes, and findings. Data synthesis was based on the PT intervention applied (e.g., therapeutic exercises, MT, and other modalities). Within each intervention category, studies were further grouped according to the type of bruxism (SB, AB, both SB and AB, or unspecified bruxism, with or without other conditions associated) and the outcomes evaluated (e.g., masticatory muscle activity, quality of life, self-reported pain, mandibular function, range of motion (ROM)). This approach facilitated the synthesis of findings despite the substantial methodological heterogeneity across studies.

Certainty of the evidence

The overall certainty of the evidence was assessed using the GRADE (Grading of Recommendations Assessment, Development and Evaluations) approach.[42] According to Guyatt et al., the certainty of evidence is classified as high, moderate, low, or very low.[42] The GRADE assessment considers the following domains: study design; risk of bias; inconsistency of results; indirectness of the evidence (not generalizable); imprecision (insufficient data); and other factors, such as reporting bias.[42]

Results

Search results

A total of 1,745 studies were identified through the database searches. Of these, 86 studies were assessed for full-text eligibility, and 13 met the inclusion criteria. The PRISMA flow diagram provides a detailed overview of the study selection process and the reasons for exclusion (Fig. 1). Additional information on excluded studies is provided in supplementary materials (Appendix 6). A manual search based on the citations of the included studies was also performed. Of the 427 records identified, 72 were duplicated and 353 were excluded after screening because they did not meet the inclusion criteria. This search yielded 2 additional eligible studies. Therefore, a total of 15 studies were included in this systematic review.

Description of the included studies

The included studies investigated different classifications of bruxism. Six studies (40.0%) included participants with SB,[28,43–46,49] one (6.7%) included participants with both AB and SB,[30] four (26.7%) did not specify the type of bruxism,[29,31,47,48] three (20.0%) included participants with unspecified bruxism associated with TMD,[13,50,51] and 1 study (6.7%) included only participants with AB.[32] Sample sizes ranged from 12 to 100 participants, and the mean age ranged from 18 to 60 years. Most studies included both male and female participants, whereas 2 studies enrolled only women.[32,43] Detailed study characteristics are presented in Tables 1 and 2.

It is important to highlight that 60% of the included studies evaluated pain as an outcome. Most participants included in these studies presented bruxism associated with orofacial pain. Seven studies (46.7%) evaluated MT,[29–32,43,49,51] including massage and conventional MT (i.e., combination of MT techniques). Three studies (20.0%) examined kinesiotaping,[28–30] 3 studies (20.0%) investigated TENS,[46,48,50] and 5 (33.3%) evaluated exercise-based interventions,[31,32,44–47] including stretching and/or active relaxation techniques such as massed negative practice (MNP), Jacobson's progressive muscle

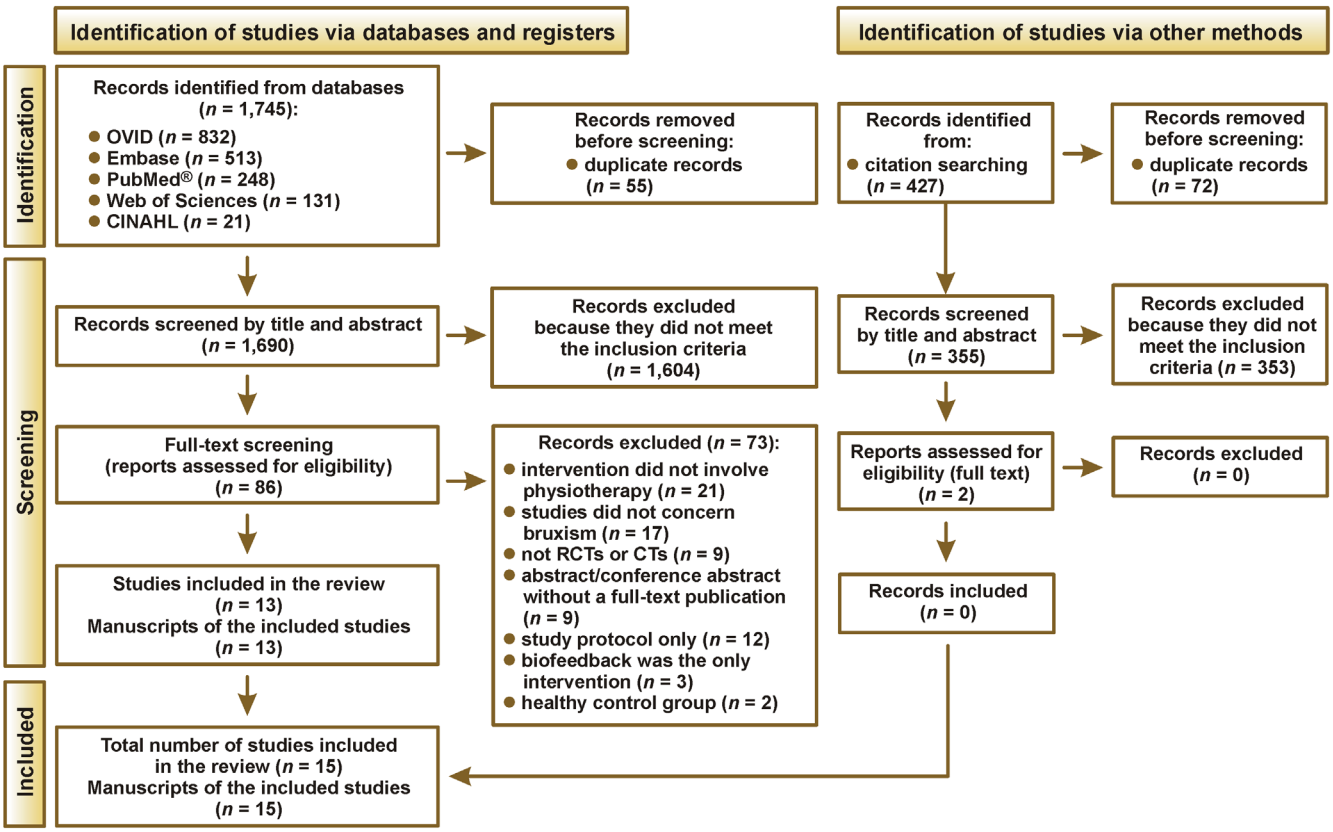


Fig. 1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flow diagram illustrating the study selection process

RCT – randomized controlled trial; CT – clinical trial.

relaxation (PMR), and muscular awareness relaxation training (MART). Three studies (20.0%) used biofeedback as a comparator intervention,[13,32,45] 1 study (6.7%) evaluated MENS,[48] and 1 study (6.7%) investigated MyoMonitor training.[13] Physiotherapy interventions were compared with several alternative treatments, including occlusal splint therapy (n = 5, 33.3%),[28,43,45,49,50] sham treatment such as sham TENS (n = 1, 6.7%) or sham biofeedback (n = 1, 6.7%),[46] and no treatment (n = 4, 26.7%).[43,47,49,51] Several outcomes were assessed using different tools. Detailed study characteristics are presented in Tables 1 and 2.

The most frequently investigated outcomes were pain (n = 9, 60%), muscle activity measured by EMG (n = 5, 33.3%), tenderness assessed using the pressure pain threshold (PPT) (n = 4, 26.7%) quality of life (n = 4, 26.7%), and mouth range of motion (ROM) (n = 4, 26.7%), among others. Outcome assessment time points varied across studies. Most studies performed evaluations immediately after treatment,[13,28–32,43,44,46–49,51] whereas others included follow-up assessments ranging from 1 to 6 months.[45,50]

Because of the considerable heterogeneity in bruxism classification, interventions and outcome measures across the included studies, a meta-analysis was not feasible. Therefore, the effects of each PT intervention compared with other treatment approaches are described below.

Interpretation of the clinical results

Manual therapy treatment

Manual therapy vs. home exercise

Kadıoğlu et al. compared the effects of MT and home exercise on orofacial pain in individuals with SB associated with headaches and parafunctional habits.[31] Pain intensity at rest, during activity and during sleep was assessed using a visual analogue scale (VAS). Both interventions reduced pain across all assessment conditions, with no significant differences between groups. However, the point estimates favored home exercise for reducing pain at rest (mean difference (*MD*) (95% confidence interval (*CI*)) = –1.84 (–3.84, 0.16)), pain during activity (*MD* (95% *CI*) = –1.45 (–3.80, 0.90)) and pain during sleep (*MD* (95% *CI*) = –1.70 (–4.32, 0.92)) (Appendix 4, Fig. A4.1, A4.2 and A4.3). According to the GRADE assessment, the certainty of the evidence for these outcomes was rated as very low (Appendix 3, Table A3).[31]

The same study also evaluated sleep quality using the Pittsburgh Sleep Quality Index (PSQI). Manual therapy resulted in significantly greater improvement than home exercise (*MD* (95% *CI*) = 3.28 points (0.67, 5.89); Appendix 4, Fig. A4.4).[31] Quality of life, assessed using the short form health survey (SF-36), improved in both groups without significant between-group

Table 1. Characteristics of the included studies (*N* = 15)

Variable		*n* (%)
Country	Turkey	5 (33.3)
	Brazil	3 (20.0)
	USA	1 (6.7)
	Austria	1 (6.7)
	India	1 (6.7)
	Netherlands	1 (6.7)
	England	1 (6.7)
	Sweden	1 (6.7)
	Germany	1 (6.7)
Publication date	before 2000	3 (20.0)
	2000–2010	4 (26.7)
	after 2010	8 (53.3)
Study design	RCT	15 (100.0)
Trial registered	yes	4 (26.7)
	no	2 (13.3)
	not reported	9 (60.0)
Ethical approval	yes	9 (60.0)
	not reported	6 (46.1)
Funding	not reported	10 (66.7)
	no funding	3 (30.0)
	government	2 (13.3)
Total sample size	≤50	11 (73.3)
	>50 but <100	4 (26.7)
Number of interventions	4	2 (13.3)
	3	2 (13.3)
	2	11 (73.3)
Bruxism classification tool*	VAS (subject-based)	4 (26.7)
	questionnaire based on the classification (subject-based)	2 (13.3)
Physiotherapy modalities*	manual therapy (massage therapy)	7 (46.7)
	kinesiotaping	3 (20.0)
	TENS	3 (20)
	exercise	5 (33.3)
	MENS	1 (6.7)
	MyoMonitor training	1 (6.7)
	BNE	1 (6.7)
Other treatment*	occlusal splint	5 (33.3)
	occlusal adjustment	1 (6.7)
	sleep hygiene advice	1 (6.7)
Sham/No treatment*	sham TENS	1 (6.7)
	no treatment	4 (26.7)
Bruxism classification	AB and SB (together)	1 (6.7)
	AB	1 (6.7)
	SB	6 (40.0)
	unspecified bruxism combined with TMD	3 (20.0)
	unspecified bruxism	4 (26.7)
Outcomes*	pain	9 (60.0)
	bruxism/EMG activity	6 (40.0)
	quality of life	4 (26.7)
	ROM	4 (26.7)
	stress level	1 (6.7)
	jaw disability	1 (6.7)
	neck disability	1 (6.7)
	EMG/PSG (device-based)	5 (33.3)
	self-reported (subject-based)	3 (20.0)

RCT – randomized controlled trial; TENS – transcutaneous electrical neuromuscular stimulation; TMD – temporomandibular disorders; ROM – range of motion; EMG – electromyography; VAS – visual analog scale; MENS – microcurrent electrical neuromuscular stimulation; BNE – bruxism neuroscience education; AB – awake bruxism; SB – sleep bruxism; PSG – polysomnography; * numbers do not add up since several studies were included in these categories.

Table 2. Summary of the interventions, comparators, outcomes, and main findings of the included studies

Study details	Participants	Intervention group
Author: Gouw et al.[44] Year: 2018 Design: RCT Objective: To determine the effectiveness of stretching of the masticatory muscles for SB Funding: Independent grant from TMJ Solutions BV Setting: Universities and University Medical Centres of Nijmegen and Utrecht; waiting rooms of several general practitioners, dentists and physical therapists	*N* = 24 (M = 9; F = 15) Age: 34.6 (9.1) years (stretching: 34.1 (6.39) years; control: 35.1 (11.5) years) Bruxism classification: SB Evaluation tool: Device-based assessment – PSG (>4 bruxism episodes/h of sleep or >25 bruxism bursts/h of sleep) Additional symptoms: None	Muscle-stretching exercises + sleep hygiene advice (MS) (*n* = 12) Description: Static masticatory muscle-stretching exercises with a stretching device Duration of the session: Unclear Duration of the treatment: Unclear Number of sessions: 10 Times/week: 2 Time point/Follow-up: End of treatment
Author: Capellini et al.[51] Year: 2006 Design: RCT Objective: To study the effect of massage on pain relief and/or EMG changes in individuals with bruxism Funding: Fundacao de Amparo a Pesquisa do Estado de São Paulo (FAPESP - protocol: 0214144/0) Setting: Faculty of Science and Technology, State University of São Paulo	*N* = 12 (M = 2; F = 10) Age: 19–22 years (MT: 20.3 (1.2) years; CG: 20.5 (1.4) years) Bruxism classification: Unspecified bruxism combined with TMD Other associated diagnosis: Myogenic TMD Evaluation tool: Clinically based assessment/subject-based assessment Questionnaire: Clinical evaluation (only TMD) Additional symptoms: Masticatory muscle pain	Massage therapy (MT) (*n* = 6) Description: Massage on the masseter and anterior temporalis. The technique was based on classic massage: superficial and deep sliding, friction, compressive stationary and with distension kneading, rolling, and superficial sliding. The intermediate substance used was mineral oil. Duration of the session: 30 min Duration of the treatment: 3 weeks Number of sessions: 15 Times/week: 5 Time point/Follow-up: The 1st EMG signal assessment occurred before the treatment and the others in the 1st, 15th and 30th days after treatment. Pain was assessed before and after the 1st and 15th massage sessions.
Author: de Paula Gomes et al.[49] Year: 2014 Design: RCT Objective: To investigate the effects of massage therapy, conventional occlusal splint therapy, and silicone occlusal splint therapy on EMG activity in the masseter and anterior temporal muscles and the intensity of signs and symptoms in individuals with severe TMD and SB Funding: NR Setting: University community of the city of São Paulo	*N* = 60 (M = 10; F = 50) Age: 18–40 years (MT: 29.32 (4.31) years; COSG: 27.89 (5.82) years; MCOSG: 26.05 (3.32) years; SOSG: 28.92 (6.78) years) Bruxism classification: SB Other associated diagnosis: Severe TMD Evaluation tool: Clinically based assessment/subject-based assessment – clinical assessment (criteria of the American Academy of Sleep Medicine). The Fonseca Anamnestic Index was used to diagnose the presence and intensity of TMD Additional symptoms: Severe TMD	Massage therapy (MT) (*n* = 15) Description: Sliding and kneading maneuvers of the masseter and anterior temporal muscles, bilaterally. A facial massage cream was used to facilitate the manual procedures. Duration of the session: 30 min Duration of the treatment: 4 weeks Number of sessions: 12 Times/week: 3 Time point/Follow-up: End of treatment

Comparison group(s)	Outcome/measurements	Results
Control group (sleep hygiene advice) (CO) (n = 12) Description: Patients received advice to control the symptoms before sleep. Practices and habits that can promote a good sleep quality, to rule out non-specific aspects. Duration of the session: NA Duration of the treatment: NA Number of sessions: NA Times/week: NA Time point/Follow-up: End of treatment	(1) Bruxism episodes [h/sleep]: Ambulant polysomnography (2) Bruxism bursts [h/sleep]: Ambulant polysomnography (3) Sleep duration [min]: Ambulant polysomnography (4) Sleep quality: 15-item validated sleep quality scale (5) MMO pain-free [mm]: ruler (6) Maximal voluntary bite force: Measured using a modified device developed by Radboudumc (6) Tenderness [kg/cm^2]: Masseter PPT measured with the Wagner algometer (7) Mandibular function: MFIQ (8) Disability: Graded Chronic Pain Scale (GCPS)	(1) Bruxism episodes: MS vs. CO Increased in both groups compared to baseline, but more in the intervention group (within-group analysis); however, there was moderate evidence against the null hypothesis between groups, slightly favoring the control group, which presented fewer bruxism episodes (p = 0.066). (2) Bruxism bursts: MS vs. CO Increased in both groups, but more in the intervention group; however, there was only moderate evidence against the null hypotheses between groups, slightly favoring the control group, which presented fewer bruxism bursts (p = 0.049). (3) Sleep duration: MS vs. CO Increased in the intervention group (within-group analysis); weak evidence against the null hypothesis, with no difference between the groups (p = 0.646). (4) Sleep quality: MS vs. CO Weak evidence against the null hypothesis, with no difference between the groups (p = 0.923). (5) Pain-free MMO: MS vs. CO Statistically significant differences between the groups, favoring the intervention group (p = 0.020). (6) Tenderness: MS vs. CO Statistically significant differences between the groups, favoring the MS group (p = 0.036). (7) MFIQ: MS vs. CO Weak evidence against the null hypothesis, with no difference between the groups (p = 0.787).
Control group (CG) (n = 6) Description: No treatment Duration of the session: NA Duration of the treatment: NA Number of sessions: NA Times/week: NA Time point/Follow-up: The 1st EMG signal assessment occurred before the treatment and the others on the 1st, 15th, and 30th days after treatment. Pain was assessed before and after the 1st and 15th massage sessions.	(1) Pain [points]: VAS (2) Masseter and temporalis activity a) Mandibular rest position (MRP): EMG activity (RMS) b) MVC: EMG activity (RMS)	(1) Pain MT vs. CG No statistically significant differences between the groups (p = NR). Within-group analysis The MT group improved after treatment (p = 0.047). (2) Masseter and temporalis activity a) Mandibular rest position (MRP) MT vs. CG No statistically significant differences between the groups (p = NR).
Conventional occlusal splint group (COSG) (n = 15) Description: After a clinical examination by a dentist, the upper arch of each volunteer was molded with irreversible hydrocolloid for the fabrication of a Michigan-type OS with canine and protrusive guides as well as a flat occlusal surface for contact with the antagonist teeth. Duration of the session: During sleeping Duration of the treatment: 4 weeks Number of sessions: NA Times/week: Everyday Follow-up: End of treatment Massage therapy + Conventional occlusal splint group (MCOSG) (n = 15) Description: Combined treatment as described in the other 2 groups (MT + COSG) Follow-up: End of treatment Silicone occlusal splint group (SOSG) (n = 15) Description: The treatment was conducted with a silicone splint made with a 3-mm soft polyvinyl sheet in a vacuum pressure molding device with a thermally controlled infrared heater. Duration of the session: During sleeping Duration of the treatment: 4 weeks Number of sessions: NA Times/week: Everyday Follow-up: End of treatment	(1) Severity of TMD [points]: Fonseca Anamnestic Index (2) Bruxism activity measured by masseter and temporalis activity (MVC) [Hz]: EMG activity	(1) Fonseca Anamnestic Index MT vs. COSG No statistically significant differences between the groups (p = NR). MT vs. MCOSG The MCOSG showed better results after treatment compared to MT (p < 0.0001). MT vs. SOSG No statistically significant differences between the groups (p = NR). COSG vs. MCOSG The MCOSG showed better results after treatment compared to COSG (p < 0.0001). COSG vs. SOSG No statistically significant differences between the groups (p = NR). MCOSG vs. SOSG The MCOSG showed better results after treatment compared to SOSG (p < 0.0001). (2) Bruxism activity measured by EMG MT vs. COSG No statistically significant differences between the groups (p = NR). MT vs. MCOSG The MCOSG showed better results after treatment compared to MT (p < 0.0001). MT vs. SOSG No statistically significant differences between the groups (p = NR). COSG vs. MCOSG The MCOSG showed better results after treatment compared to COSG (p < 0.0001). COSG vs. SOSG No statistically significant differences between the groups (p = NR). MCOSG vs. SOSG The MCOSG showed better results after treatment compared to SOSG (p < 0.0001).

Study details	Participants	Intervention group
Author: Wieselmann-Penkner et al.[13] Year: 2001 Design: RCT Objective: To compare EMG BF and TENS stimulation Funding: NR Setting: Department of Prosthodontics at the University Hospital of Graz	*N* = 20 (M = 7; F = 13) Age: 22–58 years Bruxism classification: Unspecified bruxism Evaluation tool: NR Additional symptoms: Pain and tenderness to palpation of the masticatory muscles with a chronicity of at least 6 months	MyoMonitor training (MM) (*n* = 10) Description: Two active electrodes as well as 1 reference electrode were placed on both pre-auricular regions as well as on the neck, as recommended by Jankelson (1972). In addition, surface electrodes and a multisensory electrode were attached in order to record EMG activity. Following a 5 min warming-up period (facial twitching), the current was gradually increased until the mandible performed symmetrical contractions and was left at this setting throughout a 20 min-period of rest. Duration of the session: 20 min Duration of the treatment: NR Number of sessions: 3 Times/week: 3 Time point/Follow-up: After all treatment sessions
Author: Heller and Forgione[47] Year: 1975 Design: RCT Objective: To use Forgione's method to assess the effectiveness of 2 psychological techniques in controlling bruxism – massed negative practice and (J)PMR Funding: National Institute of Dental Research Grant DE 03968-01 Setting: NR	*N* = 27 (M = 13; F = 14) + 7 non-bruxers (M = 6; F = 1) Age: 18–32 years Bruxism classification: Unspecified bruxism Evaluation tool: Clinically based assessment/subject-based assessment – self-reported (report of fatigue, tension or pain in the masticatory muscles or a report of being overheard grinding the teeth during the previous 2 months) and clinical assessment (clinical and radiographic examinations) Additional symptoms: None	Massed negative practice (MNP) (*n* = 9) Description: Participants were instructed to clench their teeth as hard as possible for five seconds and then relax the jaw for five seconds. This sequence was to be continued for a full minute and repeated five times throughout the day. Duration of the session: 5 min Duration of the treatment: 2 weeks Number of sessions: 14 Times/week: 7 Time point/Follow-up: End of treatment
Author: Pierce and Gale[45] Year: 1988 Design: RCT Objective: To compare 4 bruxism treatments for short- and long-term effectiveness: NBF, DBF, MNP, and splint therapy Funding: USPHS Research Grants DE-05344 and DE-04358 from the National Institute of Dental Research, National Institutes of Health Bethesda, MD 20892 Setting: The greater Buffalo metropolitan area	*N* = 100 (M = 35; F = 65) Age: 38 years Bruxism classification: SB Evaluation tool: Device-based – EMG (mean bruxing episodes per hour was greater than 1.0) Additional symptoms: None	Massed negative practice (MNP) (*n* = 20) Description: Was individually tailored to each of the 20 participants with bruxism in this group. Six blocks of clenching trials per day consisted of five purposefully executed clench/relax cycles varying between 5 s and 1 min. Duration of the session: 5 minutes (6 times/day) Duration of the treatment: 2 weeks Number of sessions: 14 Times/week: 7 Time point/Follow-up: End of treatment and 6 months

Comparison group(s)	Outcome/measurements	Results
Biofeedback (BF) (n = 10) Description: Two active electrodes as well as 1 reference silver chloride surface electrode were placed bilaterally on the belly of the masseter, on the pars anterior of the temporalis muscles and on the nasion. Then the patients were asked to relax and to reduce EMG activity of the masticatory muscles with the higher baseline level for 20 min (10 min masseter muscle/10 min temporalis muscle with a pause of 3 min) with the help of visual feedback. Duration of the session: 20 min Duration of the treatment: NR Number of sessions: 3 Times/week: 3 Follow-up: After all treatment sessions	(1) Bruxism activity [Hz]: EMG activity (2) Skin conductive level (SCL) [Hz]: EMG activity	(1) Bruxism activity MM vs. BF Significant reduction in bruxism activity in the MM group for the right masseter (p = 0.05). No differences between the groups (p = NR). (2) SCL MM vs. BF For both groups, MM (p = 0.004) and BF (p = 0.025), the increase was statistically significant only during the first session. No differences between the groups (p = NR).
Relaxation training (RT) (n = 6) Description: Instructions in (J)PMR were provided. Participants were given a standardized 30 min, abbreviated, relaxation training tape and instructed to practice nightly as close to bedtime as possible. Duration of the session: 30 min Duration of the treatment: 2 Number of sessions: 14 Times/week: 7 Follow-up: End of treatment Bruxer control group (CG) (n = 8) Description: No treatment Duration of the session: NA Duration of the treatment: NA Number of sessions: NA Times/week: NA Follow-up: End of treatment Healthy controls (no treatment) (n = 7)	(1) Bruxism score: Bruxism-monitoring device (2) Measuring trait of anxiety (TMAS): Taylor Manifest Anxiety Scale (3) Measuring state of anxiety: Today Form of the Multiple Affect Adjective Checklist	(1) Bruxism score MNP vs. RT vs. CG No statistically significant differences between the groups (p = NR). (2) Measuring trait of anxiety MNP vs. RT vs. CG No statistically significant differences between the groups (p = NR). (3) Measuring state of anxiety MNP vs. RT vs. CG The analysis showed significant differences in trait anxiety, but the authors did not specify between which groups. A difference was found between bruxers and non-bruxers, but not specifically between the treatment groups (p = NR).
Diurnal biofeedback (DBF) (n = 20) Description: EMG biofeedback machine was attached to the subject's masseter muscle area by means of two 10-mm-diameter gold-plated surface electrodes as active electrodes and a standard ear clip electrode attached to the ipsilateral ear as the ground. Duration of the session: 10 min Duration of the treatment: 2 weeks Number of sessions: 6 Times/week: 3 Follow-up: End of treatment and 6 months Nocturnal biofeedback (NBF) (n = 20) Description: An aversive tone sounded in an earphone when the individual bruxed. The patient was required to arise, walk across the room, and record the date and time of awakening as well as the quality of sleep. Duration of the session: Whole night Duration of the treatment: 2 weeks Number of sessions: NA Times/week: Every night Follow-up: End of treatment and 6 months Occlusal splint therapy (OS) (n = 20) Description: Flat-plane maxillary occlusal treatment splints were fabricated out of hard dental acrylic. These splints were fabricated to include full palatal coverage Duration of the session: Whole night Duration of the treatment: 2 weeks Number of sessions: NA Times/week: Everyday Follow-up: End of treatment Control group (CG) (n = 20) Description: No treatment Follow-up: End of treatment and 6 months	(1) Bruxism activity (mcVolt): (a) Bruxing episodes per hour (EMG frequencies): EMG recording during sleeping hours. (b) Duration of bruxing activity per hour (EMG durations): EMG recording during sleeping hours	(1) Bruxism activity: (a) Bruxing episodes per hour NBF and OS caused a significant reduction (p < 0.001) in EMG-measured bruxing episodes per hour, but the subsequent removal of NBF was associated with a significant increase (p < 0.01) in EMG frequencies to baseline levels. Simple main effects for EMG-measured frequencies were non-significant for MNP, DBF, and the CG. No significant differences between the groups were reported. (b) Duration of bruxing activity per hour Both NBF and OS were associated with significant decreases in EMG-measured duration of bruxing activity per hour from baseline to treatment (p < 0.01). There was also a significant increase in EMG-measured duration of bruxing activity from treatment to post-treatment for both NBF (p < 0.001) and OS (p < 0.05). Simple main effects for EMG durations over the pre-treatment, treatment, and post-treatment dimension were non-significant for MNP, DBF, and CG. No significant differences between the groups were reported.

Study details	Participants	Intervention group
Author: Treacy[46] Year: 1999 Design: RCT Objective: To test the effectiveness of relaxation training and TENS stimulation in the treatment of bruxism Funding: NR Setting: NR	*N* = 24 (M = 14; F = 10) Age: 25.1 years Bruxism classification: SB Other associated diagnosis: CMD Evaluation tool: Clinically based assessment/subject-based assessment – self-reported (report of recurring episodes of nocturnal bruxism) and clinical assessment (dental wear, muscular tenderness and ROM according to the criteria set up by Phillips et al. (1989) Additional symptoms: Symptoms of CMD	TENS (*n* = 7) Description: TENS treatment was applied to the masseter muscle to receive receive a slight, rhythmic, electrical impulse through the nerves that controls the muscles of facial expression and chewing. The TENS apparatus was attached bilaterally by wires directly over the subject's masseter muscles. A low-rate TENS (1–4 Hz) was applied. The pulse was emitted once every 1.5 s. The intensity was increased to the point where visible movement of the jaw occurred. Duration of the session: 20–30 min Duration of the treatment: 16 weeks Number of sessions: 28 Times/week: 2 Time point/Follow-up: End of treatment
Author: Doğu et al.[50] Year: 2009 Design: RCT Objective: To compare the efficacy of occlusal splint and TENS treatments on clinical findings and pain curvature in patients with myofascial pain syndrome and temporomandibular joint discomfort accompanying bruxism Funding: NR Setting: Faculty of Medicine, Department of Physical Medicine and Rehabilitation, University of Istanbul	*N* = 30 (M = 2; F = 28) Age: 33.80 (11.64) (TENS: 36.29 (13.7); OS: 31.63 (9.42)) Bruxism classification: Unspecified bruxism Other associated diagnosis: Myogenic TMD Evaluation tool: Clinically based assessment/subject-based assessment – self-reported (dental impingement complaint noticed by the patient or his relatives) and clinical assessment (detection of dental abrasions) Additional symptoms: Myofascial pain syndrome	Conventional TENS (*n* = 15) Description: TENS frequency was set at 70–110 Hz, and current duration was set as 40–100 microseconds. Electrodes were placed in the trigger points. Duration of the session: 30 min Duration of the treatment: 4 weeks Number of sessions: 20 Times/week: 5 Time point/Follow-up: End of treatment and 1 month after the end of treatment
Author: de Paula Gomes et al.[43] Year: 2015 Design: RCT Objective: To investigate the effects of massage therapy on the masticatory muscles and occlusal splint usage, quality of life and pain in individuals with SB Funding: NR Setting: University community	*N* = 100 (F = 100) (final sample of 78) Age: 18–40 years (MT: 24.4 (4.1) years; OS: 24.4 (4.1) years; MT+OS: 28.6 (4.2) years; CG: 25.72 (6.2) years) Bruxism classification: SB Evaluation tool: Clinically based assessment/subject-based assessment – clinical assessment (criteria of the American Academy of Sleep Medicine) Additional symptoms: Pain intensity score of 3 on a 11-point NRS.	Massage therapy (MT) (*n* = 17) Description: Massage of masticatory muscles. Sliding and kneading maneuvers of the masseter and anterior temporal muscles, bilaterally. A facial massage cream was used to facilitate the manual procedures. Duration of the session: 30 min Duration of the treatment: 4 weeks Number of sessions: 12 Times/week: 3 Time point/Follow-up: End of treatment

Comparison group(s)	Outcome/measurements	Results
MART (n = 8) Description: The program included training in awareness of posture and breathing habits by assuming different body positions and performing various movements. In addition, various muscular relaxation awareness exercises, involving neck, eye, arm, lower back, and leg muscles were used. The subject assumed supine as well as erect positions during the program. Duration of the session: 20–30 min Duration of the treatment: 16 weeks Number of sessions: 28 Times/week: 2 Follow-up: End of treatment Sham TENS (n = 8) Description: Sham TENS treatment of the masseter muscle, e.g., TENS with no electrical impulses Duration of the session: 20–30 min Duration of the treatment: 16 weeks Number of sessions: 28 Times/week: 2 Follow-up: End of treatment	(1) Jaw ROM [mm]: Ruler (2) Number of breaths per minute: Counting (3) Bruxism activity [μV]: EMG activity (4) Discomfort in the craniomandibular area (quality of life): The subjects estimated their degree of discomfort on a scale ranging from 0 (absence of discomfort) to 6 (great discomfort)	(1) Jaw ROM TENS vs. MART Participants in the MART group showed a significantly greater increase in MMO than those in the TENS group ($p < 0.05$). TENS vs. sham TENS No significant differences between the groups ($p > 0.05$). MART vs. sham TENS Participants in the MART group showed a significantly greater increase in MMO than those in the sham TENS group ($p < 0.05$). (2) Number of breaths TENS vs. MART Participants in the MART group showed a significantly greater reduction in the number of breaths than those in the TENS group ($p < 0.05$). TENS vs. sham TENS No significant differences between the groups ($p > 0.05$). MART vs. sham TENS Participants in the MART group showed a significantly greater reduction in the number of breaths than those in the sham TENS group ($p < 0.05$). (3) Bruxism activity (M. frontalis and M. masseter) TENS vs. MART Participants in the MART group showed a significantly greater reduction in bruxism activity than those in the TENS group ($p < 0.05$). TENS vs. sham TENS No significant differences in bruxism activity between the groups ($p > 0.05$). MART vs. sham TENS Participants in the MART group showed a significantly greater reduction in bruxism activity than those in the sham TENS group ($p < 0.05$). (4) Quality of life TENS vs. MART No significant differences between the groups. TENS vs. sham TENS No significant differences between the groups. MART vs. sham TENS Participants in the MART group showed significantly better quality of life than those in the sham TENS group ($p < 0.05$).
Occlusal splint (OS) (n = 15) Description: Mandibular occlusal splint was prepared for each patient. Patients used the splint for 24 hours for a month after eating and brushing. Duration of the session: The whole day Duration of the treatment: 4 weeks Number of sessions: NA Times/week: NA Follow-up: End of treatment and 1 month after the end of treatment	(1) Pain at rest and functioning: VAS (2) Tenderness of masseter and temporalis: PPT [kg/cm^2] of the trigger points (3) Quality of life: Short Form-36 (4) ROM [mm]: Tool NR	(1) Pain TENS vs. OS Pain improved more after TENS than after OS both after treatment ($p < 0.028$) and at the 1-month follow-up ($p < 0.032$). (2) Tenderness of the masseter and temporalis TENS vs. OS No statistically significant differences between the groups (p = NR). (3) Quality of life TENS vs. OS No statistically significant differences between the groups (p = NR). (4) ROM TENS vs. OS TENS was more effective than OS ($p < 0.05$).
Occlusal splint (Michigan-type) (OS) (n = 19) Description: After a clinical examination by a dentist, the upper arch of each volunteer was molded with irreversible hydrocolloid for the fabrication of a Michigan-type OS with canine and protrusive guides as well as a flat occlusal surface for contact with the antagonist teeth. Duration of the session: During sleeping Duration of the treatment: 4 weeks Number of sessions: NA Times/week: Everyday Follow-up: End of treatment Massage therapy + occlusal splint (MT + OS) (n = 23) Description: Combined treatment as described in the other two groups (MT + COSG) Follow-up: End of treatment Control group (CG) (n = 19) Description: No treatment Duration of the session: NA Duration of the treatment: NA Number of sessions: NA Times/week: NA Follow-up: End of treatment	(1) Quality of life: SF-36 (2) Pain intensity: NRS	(1) Quality of life MT vs. MT+OS vs. OS No statistically significant differences between the groups (p = NR). (2) Pain intensity MT vs. OS No statistically significant differences between the groups (p = NR). MT vs. MT+OS The MT+OS group showed better results after treatment than the MT group ($p < 0.05$). MT vs. CG The MT group showed better results after treatment than the CG ($p < 0.0001$). OS vs. MT+OS The MT+OS group showed better results after treatment than the OS group ($p < 0.05$). OS vs. CG The OS group showed better results after treatment than the CG ($p < 0.003$). MT+OS vs. CG The MT+OS group showed better results after treatment than the CG ($p < 0.0001$).

Study details	Participants	Intervention group
Author: Volkan-Yazici et al.[30] Year: 2021 Design: RCT Objective: To compare the effects of two physiotherapy methods (manual therapy and KT with manual therapy) in patients with bruxism Funding: NR Setting: Ankara University Faculty of Dentistry	*N* = 32 (M = 2; F = 30) Age: MT: 24.6 (3.9) KTMT: 25.3 (4.1) Bruxism classification: AB and SB Evaluation tool: Clinically based assessment/subject-based assessment – self-reported and clinical assessment (Criteria of the International Classification for Sleep Disorders of the American Academy of Sleep Medicine, extracted from the protocol) Additional symptoms: None	Manual therapy (MT) (*n* = 16) Description: MT of the masticatory muscles performed intra- and extraorally together with MT of the cervical region were used in the treatment of bruxism. Stretching exercises of the masseter muscle were also applied. Extraoral applications consisted of emphasis on the masseter and temporalis muscle using kneading, friction, intramuscular stretching, sliding, and ischemic compression for a trigger point release. Myofascial release techniques were used to eliminate pain. Additionally, active and passive exercises were applied to the masseter, temporalis, digastric, medial pterygoid, and lateral pterygoid muscles. Intraoral applications consisted of emphasis on the masseter muscles using kneading, friction, intramuscular stretching and sliding, and ischemic compression for a trigger point release. Applications of the cervical region consisted of upper cervical flexion mobilization, C2–C5 central posterior-anterior mobilization, and also kneading, friction, intramuscular stretching, and sliding maneuvers for the cervical muscles. Duration of the session: 30 min Duration of the treatment: 2 weeks Number of sessions: 8 sessions Times/week: 4 Time point/Follow-up: End of the treatment
Author: Rajpurohit et al.[48] Year: 2010 Design: RCT Objective: To compare the effectiveness of TENS and MENS on masticatory muscle pain in bruxism patients Funding: NR Setting: Physiotherapy Department, KLES Prabhakar Kore Hospital and MRC, Belgaum	*N* = 60 (M = 36; F = 24) Age: 19–60 years Bruxism classification: Unspecified bruxism Evaluation tool: Clinically based assessment/subject-based assessment – clinical assessment (muscle tenderness over the masseter muscle; early morning temporomandibular joint stiffness and pain; duration of pain of more than 3 weeks). Additional symptoms: Masticatory muscle pain	TENS (*n* = 30) Description: Carbon electrodes (40 mm × 54 mm) were placed over the affected side of the masseter muscle with frequency operating at 50 Hz and the pulse width of 0.5 mSec, whereas the intensity was based on the patient's tolerance. Duration of the session: 20 min Duration of the treatment: 1 week Number of sessions: 7 Times/week: 7 Time point/Follow-up: End of treatment
Author: Yazici et al.[29] Year: 2023 Design: RCT Objective: To compare the immediate effects of a single session of manual therapy (MT) or kinesiotaping following MT (KTMT) in patients with bruxism using shear wave ultrasonography Funding: NR Setting: Ankara University Faculty of Dentistry	*N* = 39 (M = 4; F = 35) Age: MT: 27.3 (4.8) KTMT: 26.2 (5.4) Bruxism classification: AB and SB (extracted from the protocol) Evaluation tool: Clinically based assessment/subject-based assessment – self-reported and clinical assessment (Criteria of the International Classification for Sleep Disorders; extracted from the protocol) Additional symptoms: None	Manual therapy (MT) (*n* = 20) Description: MT of the masticatory muscles performed intra- and extraorally together with MT of the cervical region were used in the treatment of bruxism. Stretching exercises of the masseter muscle were also applied. Extraoral applications consisted of emphasis on the masseter and temporalis muscle using kneading, friction, intramuscular stretching, sliding, and ischemic compression for a trigger point release. Myofascial release techniques were used to eliminate pain. Additionally, active and passive exercises were applied to the masseter, temporalis, digastric, medial pterygoid, and lateral pterygoid muscles. Intraoral applications consisted of emphasis on the masseter muscles using kneading, friction, intramuscular stretching and sliding, and ischemic compression for a trigger point release. Applications of the cervical region consisted of upper cervical flexion mobilization, C2–C5 central posterior-anterior mobilization, and also kneading, friction, intramuscular stretching, and sliding maneuvers for the cervical muscles. Duration of the session: 30 min Duration of the treatment: 1 week Number of sessions: 1 session Times/week: 1 Time point/Follow-up: Immediately after the 1st session

Comparison group(s)	Outcome/measurements	Results
Kinesiotaping + manual therapy (KTMT) (n = 16) Description: The web-cut strips EDF (epidermisdermis-fascia) technique was used. KT placement was chosen according to the principles described by Kase et al. Tapes were cut 2.5 cm in width (cut in half vertically from a 5-cm width). Two strips of kinesiotape were crossed over the masseter muscle area with 0–5% tension in the maximum stretched epidermis position. The material used for bruxism KT application was Kinesio Tex Gold Fingerprints – a 100% cotton, latex-free, 5-cm wide, elastic tape. The tape was applied bilaterally after the MT session. Duration of the session: 30 min Duration of the treatment: 2 weeks Number of sessions: 8 sessions Times/week: 4 The kinesiotape was applied bilaterally after every session of MT, and the tape was removed 1 day before the next MT session. Follow-up: End of the treatment	(1) Masseter muscle stiffness [cm]: Ultrasound imaging (2) Masseter muscle thickness [m/s]: Ultrasound imaging (3) Tenderness: PPT was determined using a digital algometer; PPTs of the masseter, trapezius (anterior, posterior, occipital), and temporalis muscles were assessed bilaterally. (4) Quality of life: The factors associated with bruxism which caused problems in the daily life were assessed via Likert scale (5) Sleep Quality: PSQI (6) Pain intensity 0–5 Likert scale	(1) Muscle stiffness MT vs. KTMT Significant decreases in the masseter muscle were observed in both groups ($p < 0.05$). No differences between the groups ($p > 0.05$). (2) Muscle thickness MT vs. KTMT Significant decreases in masseter muscle thickness were observed in both groups ($p < 0.05$). No differences between the groups ($p > 0.05$). (3) Tenderness MT vs. KTMT Jaw pain decreased more in the KTMT group than in the MT group ($p < 0.05$). (4) Quality of life MT vs. KTMT Significant improvement was observed in both groups ($p < 0.05$). No differences between the groups ($p > 0.05$) (5) Sleep quality MT vs. KTMT Significant improvement was observed in both groups ($p < 0.05$). No differences between the groups ($p > 0.05$). (6) Pain intensity MT vs. KTMT Jaw pain decreased more in the KTMT group than in the MT group ($p < 0.05$).
MENS (n = 30) Description: Frequency of 0.5 Hz and intensity based on the patient's tolerance Duration of the session: 20 min Duration of the treatment: 1 week Number of sessions: 7 Times/week: 7 Follow-up: End of treatment	(1) Tenderness: Digital pressometer of about 2 KgF (2) Pain: VAS	(1) Tenderness TENS vs. MENS A significantly greater improvement in tenderness was observed with MENS than with TENS ($p < 0.0495$). (2) Pain TENS vs. MENS Pain relief was greater with MENS therapy than with TENS, with a statistically significant difference at the end of treatment ($p \leq 0.0001$) (TENS group: 3.38 (1.25) vs. MENS group: 3.91 (0.83)).
Kinesiotaping + manual therapy (KTMT) (n = 16) Description: The web-cut strips EDF (epidermisdermis-fascia) technique was used. KT placement was chosen according to the principles described by Kase et al. Tapes were cut 2.5 cm in width (cut in half vertically from a 5-cm width). Two strips of kinesiotape were crossed over the masseter muscle area with 0–5% tension in the maximum stretched epidermis position. The material used for bruxism KT application was Kinesio Tex Gold Fingerprints – a 100% cotton, latex-free, 5-cm wide, elastic tape. The tape was applied bilaterally after the MT session. Duration of the session: 45 min Duration of the treatment: 1 week Number of sessions: 1 session Times/week: 1 The kinesiotape was applied bilaterally after every session of MT, and the tape was removed 1 day before the next MT session. Follow-up: Immediately after the 1st session	(1) Masseter muscle stiffness [cm]: Ultrasound imaging (2) Masseter muscle thickness [m/s]: Ultrasound imaging (3) Tenderness: PPT was determined using a digital algometer; PPTs of the masseter, trapezius (anterior, posterior, occipital), and temporalis muscles were assessed bilaterally.	(1) Muscle stiffness MT vs. KTMT Significant decreases in the masseter muscle were observed in both groups ($p < 0.05$). No between-group differences were observed ($p > 0.05$). (2) Muscle thickness MT vs. KTMT Significant decreases in masseter muscle thickness were observed in both groups ($p < 0.05$). No between-group differences were observed ($p > 0.05$). (3) Tenderness MT vs. KTMT A significant increase in PPT for the masseter and temporalis muscles was observed in the KTMT group ($p < 0.05$). No significant differences in PPT were found for any other muscle groups in either the KTMT or MT groups ($p > 0.05$). No between-group differences were observed ($p > 0.05$).

Study details	Participants	Intervention group
Author: Keskinruzgar et al.[28] Year: 2019 Design: RCT Objective: To evaluate the efficacy of KT in patients with SB and to determine whether kinesiotaping may be an alternative for OS for the treatment of SB Funding: NR Setting: Oral and Maxillofacial Surgery Clinic at the Faculty of Dentistry of Adıyaman University	*N* = 34 (M = 4; F = 30) Age: KT: 27.38 (9.05) years; OS: 26.11 (8.71) years Bruxism classification: SB Evaluation tool: Clinically based assessment/subject-based assessment – self-reported (clenching or grinding of teeth for at least 5 days a week for duration of 6 months) and clinical assessment (presence of shiny dental restorations, dental abrasion, hypertrophy in masseter muscles and palpation-induced pain) Additional symptoms: None	Kinesiotaping (KT) (*n* = 16) Description: KT was performed by a physiotherapist as described by Dr. Kase, where the 5-cm-wide tapes were cut in the Y-shape. The ends of the Y-shaped band were attached to the region corresponding to the masseter muscle's insertion with a tension of about 40%. Duration of the session: Whole day and night Duration of the treatment: 5 weeks Number of sessions: Unclear Times/week: 7 Time point/Follow-up: After the 1st week of treatment and at the end of treatment
Author: von Piekartz et al.[32] Year: 2024 Design: RCT Objective: To assess a comprehensive PT strategy that integrates both direct (hands on) and indirect (hands off) treatments for AB, aimed to relieve pain and enhance orofacial/cervical function Funding: NR Setting: Physical therapy practices in Western Germany	*N* = 42 (F = 42) Age: PT: 31.30 (11.16) years; Control: 28.50 (10.33) years Bruxism classification: AB Evaluation tool: Clinically based – classification provided by a dentist familiar with a bruxism classification system Additional symptoms: 60% of the population presented TMD diagnosis (40% myofascial TMD; 7% arthrogenic TMD; 13% mixed TMD) with or without chronic pain	Multimodal PT program (*n* = 13) Description: Orofacial manual therapy, motor control and behavioral exercise, habitual reversal training, the brace relaxation technique, tongue-teeth breathing swallowing exercise, Brux.app (education therapy), habit formation exercise, BNE, which includes information about somatomotor reflex, nociceptor (warning signals), centralized mechanism, memory for motor functions, (un)controlled motor inhibition system, management of motion control. Duration of the session: NR Duration of the treatment: 3 weeks Number of sessions: 6 Times/week: 2 Time point/Follow-up: End of treatment
Author: Kadıoğlu et al.[31] Year: 2024 Design: RCT Objective: To examine the effects of manual therapy and home exercise treatments on pain, sleep quality, stress level, and quality of life in patients with bruxism Funding: No funding Setting: Bartın University, School of Health Services, Bartın, Türkiye	*N* = 30 (M = 9; F = 21) Age: MTG: 18–20: 6 (40%); 21–22: 7 (46.7%); 23–up: 2 (13.3%); HEG: 18–20: 7 (46.7%); 21–22: 4 (26.6%); 23–up: 4 (26.6%); Bruxism classification: Unspecified bruxism Evaluation tool: Subject-based – individuals who were pre-classified with "bruxism" in the preliminary evaluation of the questionnaire were clinically evaluated by an expert orthodontist in terms of clinical signs of bruxism: abnormal tooth wear on the occlusal surfaces of the teeth, abfraction, gingival recession and/or cervical defect, tongue indentations, damage to the inside of the cheek, tense facial and jaw muscles, muscle sensitivity, and masseteric hypertrophy upon bidigital palpation. Individuals who had at least two of these clinical findings in addition to the bruxism questionnaire results were included in the study. Additional symptoms: 86.7% of the population presented with associated headache. All participants present parafunctional habits	Manual therapy group (MTG) (*n* = 15) Description: This group involves manual therapy for jaw and neck muscles including soft tissue, joint mobilization, intramuscular stretches, trigger point therapy, intraoral application, post-isometric relaxation. Additionally, stretching, relaxation and strengthening training. Duration of the session: 40 min Duration of the treatment: 8 weeks Number of sessions: 16 Times/week: 2 Time point/Follow-up: End of treatment

BF – biofeedback; CG – control group; CMD – craniomandibular dysfunction; DBF – diurnal biofeedback; F – female; (J)PMR – Jacobson's progressive muscle relaxation; KT – kinesiotaping; KTMT – kinesiotaping and manual therapy; M – male; MART – muscular awareness relaxation training; MFIQ – Mandibular Function Impairment Questionnaire; MNP – massed negative practice; MM – MyoMonitor training; MMO – maximal mouth opening; MT – massage therapy; MVC – maximal voluntary contraction; NA – not applicable; NBF – nocturnal biofeedback; NR – not reported; NRS – numeric rating scale; OS – occlusal splint; PPT – pain pressure threshold; PSQI – Pittsburgh Sleep Quality Index; RMS – root mean square; RT – relaxation training; SOSG – silicone occlusal splint group; QoL – quality of life.

Comparison group(s)	Outcome/measurements	Results
Occlusal splint (OS) (n = 18) Description: Models were prepared by using the upper jaw measurements of the patients. For each patient, 0.5-mm thick thermoplastic hard splints were fabricated as OS by using these models. Duration of the session: whole night Duration of the treatment: 5 weeks Number of sessions: NA Times/week: Every night Follow-up: After the 1st week of treatment and at the end of treatment	(1) ROM: Maximum distance between the patient's lower and upper incisors (tool NR) (2) Pain: VAS (3) Tenderness: Bilateral temporalis and masseter muscle PPTs were measured with an algometer (J Tech Digital Algometer)	(1) ROM KT vs. OS No statistically significant differences between the groups ($p > 0.05$). (2) Pain KT vs. OS No statistically significant differences between the groups ($p > 0.05$). (3) Tenderness KT vs. OS No statistically significant differences between the groups ($p > 0.05$), except for the temporalis PPT at the 1st week, which was significantly higher in the KT group than in the OS group ($p = 0.03$).
Control group (CG) (n = 15) Description: Waiting list Duration of the session: NA Duration of the treatment: NA Number of sessions: NA Times/week: NA Follow-up: End of treatment	(1) ROM: Mouth opening, propulsion, retropulsion, laterotrusion (2) Perception of functional limitations due to pain-related problems in daily life: The Pain Disability Index (PDI) (3) Neck-related disability: Neck Disability Index (NDI) (4) Jaw disability: Jaw Disability List (JDL) (5) Mechanical pressure pain threshold (PPT): masseter	(1) ROM Mouth opening: No differences between the groups were observed (*MD*: −4.18 (95% *CI*: −11.28, 2.92)) Propulsion: No differences between the groups were observed (*MD*: 0.44 (95% *CI*: −1.56, 2.44)) Retropulsion: No differences between the groups were observed (*MD*: 0.20 (95% *CI*: −1.51, 1.91)) (2) Perception of functional limitations due to pain-related problems in daily life: No differences between the groups were observed (*MD*: 4.88 (95% *CI*: −3.52, 13.28)) (3) Neck-related disability: PT resulted in greater improvement in the NDI than HEG (*MD*: 6.94 (95% *CI*: −1.51, 15.39)) (4) Jaw disability: Results NR (5) Mechanical pressure pain threshold (PPT): Results NR
Home exercises (HEG) (n = 15) Description: This group involved home exercises for jaw and neck muscles, including mobility, stretching, isometric, posterior tilt, postural exercises, as well as relaxation and strengthening training. Duration of the session: 25 min Duration of the treatment: 8 weeks Number of sessions: NA Times/week: NA Follow-up: End of treatment	(1) Sleep quality: PSQI (2) QoL: SF-36 (3) Stress level: Perceived Stress Scale (4) Jaw disability: Fonseca Anamnestic Index (5) Number of TrP (6) Rest pain: VAS (0–10) (7) Activity pain: VAS (0–10) (8) Night pain: VAS (0–10)	(1) Sleep quality: MTG improved sleep quality compared with HEG (*MD*: 3.28 (95% *CI*: 0.67, 5.89)) (2) Quality of life: MTG improved quality of life compared with HEG (*MD*: −15.66 (95% *CI*: −32.17, 0.85)) (3) Stress level: no differences between the groups were observed (*MD*: 1.47 (95% *CI*: −1.97, 4.91)) (4) Jaw disability: no differences between the groups were observed (*MD*: 6.34 (95% *CI*: −5.42, 18.10)) (5) Number of TrPs: MTG had fewer TrPs than HEG (*MD*: 2.07 (95% *CI*: 0.16, 3.98)) (6) Rest pain: no differences between the groups were observed (*MD*: −1.84 (95% *CI*: −3.84, 0.16)) (7) Activity pain: no differences between the groups were observed (*MD*: −1.45 (95% *CI*: −3.80, 0.90)) (8) Night pain: no differences between the groups were observed (*MD*: −1.70 (95% *CI*: −4.32, 0.92))

differences, although the point estimates favored MT (*MD* (95% *CI*) = –15.66 points (–32.17, 0.85); Appendix 4, Fig. A4.5). Similarly, stress levels assessed using the perceived stress scale (*MD* (95% *CI*) = 1.47 points (–1.97, 4.91); Appendix 4, Fig. A4.6) and jaw function assessed using the Fonseca Anamnestic Index (FAI) (*MD* (95% *CI*) = 6.34 points (–5.42, 18.10); Appendix 4, Fig. A4.7), as well as the number of trigger points improved in both groups, without any significant differences noted. In contrast, MT resulted in significantly fewer trigger points (*MD* (95% *CI*) = 2.07 points (0.06, 3.98); Appendix 4, Fig. A4.8). The certainty of the evidence for all outcomes was rated as very low according to the GRADE assessment (Appendix 3; Table A3).[31]

Manual therapy vs. manual therapy + kinesiotaping

Volkan-Yazici et al. compared MT alone to MT combined with kinesiotaping in individuals with both SB and AB associated with orofacial pain. Pain intensity was measured using a Likert scale.[30] Both groups demonstrated improvements in pain intensity following treatment, and jaw pain decreased more in the KTMT group compared with the MT group ($p < 0.05$; (*MD* (95% *CI*) = –0.34 points (–0.90, 0.22); Appendix 4, Fig. A4.9). The certainty of the evidence was rated as very low according to GRADE (Appendix 3, Table A3).

One study reported in 2 publications[29,30] compared MT alone to MT combined with kinesiotaping in individuals with both SB and AB presenting tenderness of the orofacial and cervical muscles. Tenderness was assessed bilaterally in the masseter, temporalis and trapezius muscles. No significant differences between the groups were observed in tenderness reduction (Appendix A4, Fig. A4.10). The certainty of the evidence was rated as very low (Appendix 3, Table A3).

Manual therapy vs. occlusal splint therapy

De Paula Gomes et al. compared 4 treatment groups: massage therapy; occlusal splint therapy; massage combined with occlusal splint therapy; and no treatment in individuals with SB associated with TMD.[43] Orofacial pain intensity was measured using the numeric rating scale (NRS) after 4 weeks of treatment. Combined therapy (massage plus occlusal splint therapy) produced significantly greater reductions in pain intensity than massage alone (*MD* (95% *CI*) = –2.51 points (–3.57, –1.45)), occlusal splint therapy alone (*MD* (95% *CI*) = 2.37 points (1.37, 3.37)), and no treatment (*MD* (95% *CI*) = 4.41 points (3.55, 5.27)). Massage therapy alone also resulted in greater pain reduction than no treatment (*MD* (95% *CI*) = 1.90 points (0.90, 2.90)). However, no significant differences were observed between massage therapy and occlusal splint therapy alone (*MD* (95% *CI*) = –0.14 points (–1.26, 0.98)) (Appendix A4; Fig. A4.11). The certainty of the evidence for all comparisons was rated as very low according to GRADE (Appendix 3, Table A3).[43]

The same research group evaluated bruxism activity by measuring maximal voluntary contraction (MVC) of the masseter and temporalis muscles using EMG.[49] The authors reported greater reductions in bruxism activity following combined treatment (massage + occlusal splint therapy) than after either massage therapy or occlusal splint therapy alone. However, numerical data were not reported, so the data could not be represented in a forest plot. The certainty of the evidence was rated as very low (Appendix 3, Table A3).[49]

Manual therapy vs. no treatment

Capellini et al. compared massage therapy with no treatment in individuals with unspecified bruxism associated with TMD.[51] Bruxism activity was evaluated by measuring the root mean square of the EMG signal at the mandibular rest position (RMS-MRP) of the masseter and temporalis muscles.[51] No significant between-group differences were observed in RMS-MRP for any of the 4 muscles evaluated across the 4 EMG assessment sessions. The authors also reported reductions in orofacial pain following massage therapy compared with no treatment. However, numerical outcome data was not provided; therefore, quantitative synthesis was not possible. The certainty of the evidence was rated as very low according to GRADE (Appendix 3, Table A3).[51]

Stretching

Muscle active stretching vs. sleep hygiene advice (control group)

Gouw et al. compared active stretching combined with sleep hygiene advice with sleep hygiene advice alone in individuals with SB without any additional symptoms.[44] Bruxism activity was divided into subcategories based on the frequency of bruxism episodes (episodes/hour of sleep) and bruxism bursts (bursts/hour of sleep). Mouth ROM was evaluated using 3 measures: active maximal mouth opening (MMO), passive MMO and pain-free MMO. Tenderness was assessed using PPT. Neither intervention produced significant reductions in the frequency of bruxism bursts or episodes, and no significant differences were observed between the groups following treatment (Appendix A4, Fig. A4.12). Similarly, no significant between-group differences were found for active MMO (*MD* (95% *CI*) = 1.30 mm (–6.79, 9.39)), pain-free MMO (*MD* (95% *CI*) = 3.20 mm (–5.73, 12.13)) and passive MMO (*MD* (95% *CI*) = –0.20 mm (–8.03, 7.63); Appendix A4, Fig. A4.13). In contrast, participants receiving active stretching combined with sleep hygiene advice demonstrated significantly greater improvements in tenderness than those receiving sleep hygiene advice alone (*MD* (95% *CI*) = 1.00 kg/cm^2 (0.05, 1.95); Appendix A4, Fig. A4.14); According to the GRADE assessment, the certainty of the evidence for all outcomes was rated as very low (Appendix 3, Table A3).[44]

Exercise therapy

MyoMonitor training vs. biofeedback training

Wieselmann-Penkner et al. compared MyoMonitor training with biofeedback training in individuals with unspecified bruxism associated with tenderness of the masticatory muscles.[13] Electromyographic activity of the right and left masseter and temporalis muscles was measured weekly throughout the whole treatment period and after each of the 3 treatment sessions. Overall, no significant between-group differences were observed after the first or third treatment sessions for any of the muscles evaluated (Appendix A4, Fig. A4.15,A4.17). However, following the second treatment session, participants in the MyoMonitor group demonstrated significantly greater reductions in bruxism activity for both the right masseter (*MD* (95% *CI*) = –2.33 (–4.53, –0.13)) and the left masseter (*MD* (95% *CI*) = –2.22 (–4.27, –0.17)) compared to those in the biofeedback group (Appendix A4, Figure A4.16). The certainty of the evidence for all comparisons was rated as very low according to the GRADE assessment (Appendix 3, Table A3).

Massed negative practice vs. occlusal splint therapy + strength exercise + biofeedback

Pierce and Gale compared MNP with occlusal splint therapy, strength exercise, nocturnal biofeedback, and diurnal biofeedback in individuals with SB without associated symptoms. Bruxism activity was assessed by the frequency of bruxism episodes per hour of sleep (EMG frequency) and the duration of bruxism activity per hour of sleep (EMG duration).[45] No significant differences were observed among the treatment groups in either outcome at the end of the treatment or at the one-month follow-up. Numerical data was not reported; therefore, the results could not be represented in a forest plot. The certainty of the evidence was rated as very low according to the GRADE assessment (Appendix 3, Table A3).[45]

Massed negative practice vs. education therapy (Jacobson's progressive muscle relaxation) and no treatment

Heller and Forgione compared MNP with education therapy based on Jacobson's PMR and with no treatment in individuals with unspecified bruxism without associated symptoms.[47] No significant differences in bruxism activity were observed among the groups at the end of the treatment or at the one-month follow-up (Appendix A4, Fig. A4.18,A4.19). The certainty of the evidence was rated as very low according to the GRADE assessment (Appendix 3, Table A3).[47]

Kinesiotaping

Kinesiotaping vs. occlusal splint therapy

Keskinruzgar et al. compared kinesiotaping with occlusal splint therapy in individuals with SB without associated symptoms. The outcomes evaluated were pain intensity, muscle tenderness and mouth ROM.[28] No significant between-group differences were observed for pain intensity, although the point estimates slightly favored kinesiotaping (*MD* (95% *CI*) = 1.40 points (–0.34, 3.14)) (Appendix A4; Fig. A4.20). Similarly, no significant between-group differences were found for tenderness of either the masseter muscle (*MD* (95% *CI*) = –1.43 (–4.14, 1.28)) (Appendix A4; Fig. A4.21) or the temporalis muscle (*MD* (95% *CI*) = –2.13 (–5.75, 1.49)) (Appendix A4; Fig. A4.22), as well as for active MMO (*MD* (95% *CI*) = –0.88 mm (–6.47, 4.71)) (Appendix A4, Fig. A4.22). The certainty of the evidence for all outcomes was rated as very low according to the GRADE assessment (Appendix 3, Table A3).

Transcutaneous electrical nerve stimulation (TENS)

TENS vs. sham TENS vs. muscular awareness relaxation training

Treacy compared TENS, sham TENS and MART, an active relaxation technique, in individuals with SB associated with symptoms of craniomandibular disorders.[46] Participants in the MART group demonstrated significantly greater improvements in mouth ROM than those receiving either active or sham TENS ($p < 0.05$). No significant differences were observed between TENS and sham TENS groups ($p > 0.05$). Bruxism burst was measured using EMG recordings of the frontalis and masseter muscles at rest [μV]. Participants receiving MART demonstrated significantly greater reductions in bruxism activity than those receiving TENS ($p < 0.05$), whereas no significant differences were observed between active and sham TENS ($p > 0.05$). Numerical outcome data was not reported; therefore, the results could not be presented in a forest plot. The certainty of the evidence was rated as very low according to the GRADE assessment (Appendix 3, Table A3).[46]

TENS vs. occlusal splint therapy

Doğu et al. compared the effect of TENS with occlusal splint therapy in individuals with unspecified bruxism associated with TMD and myofascial pain.[50] The outcomes evaluated included pain intensity, muscle tenderness, quality of life, and ROM. Both groups demonstrated reductions in pain intensity after treatment; however, no clinically or statistically significant differences were noted between the groups either immediately after treatment or at the one-month follow-up. Numerical outcome data was not reported; therefore, the results could not be presented in a forest plot.[50]

Muscle tenderness was assessed at 3 anatomical regions of the masseter muscle bilaterally after treatment and at the one-month follow-up. Immediately after treatment, occlusal splint therapy resulted in significantly greater improvements than TENS for the right masseter origin (*MD* (95% *CI*) = –0.90 kg/cm^2 (–1.70, –0.10)), right masseter

insertion (*MD* (95% *CI*) = −0.97 kg/cm^2 (−1.71, −0.19)), left masseter origin (*MD* (95% *CI*) = −1.60 kg/cm^2 (−2.64, −0.56)), left masseter body (*MD* (95% *CI*) = −1.05 kg/cm^2 (−1.94, −0.16)), and left masseter insertion (*MD* (95% *CI*) = −1.80 kg/cm^2 (−2.68, −0.92)) (Appendix A4, Fig. A4.23). At the one-month follow-up, no significant differences were noted between the groups (Appendix A4, Fig. A4.24).

Quality of life was evaluated using 4 domains of the SF-36 questionnaire. No significant differences were observed between the groups immediately after treatment or at the one-month follow-up (Appendix A4, Fig. A4.25,A4.26). According to the GRADE assessment, the certainty of the evidence for these outcomes was rated as low (Appendix 3, Table A3).

TENS vs. MENS

Rajpurohit et al. compared MENS with TENS in individuals with unspecified bruxism associated with masticatory muscle pain.[48] Pain intensity was the primary outcome. The authors reported significantly greater reductions in pain intensity and muscle tenderness following MENS than TENS ($p < 0.0495$). However, numerical outcome data was not provided; thus, quantitative analysis was not possible. The certainty of the evidence was rated as very low according to the GRADE assessment (Appendix 3, Table A3).[48]

Multimodal treatment

Multimodal treatment vs. waiting list

Von Piekartz et al. compared a multimodal PT program consisting of MT, exercise, biofeedback, and education with a waiting-list control group in individuals with AB associated with TMD.[32] No significant between-group differences were observed following treatment for mouth opening (*MD* (95% *CI*) = −4.18 mm (−11.28, 2.92)), mandibular protrusion (*MD* (95% *CI*) = 0.44 mm (−1.56, 2.44)), or mouth lateral deviation (*MD* (95% *CI*) = 0.20 mm (−1.51, 1.91)) (Appendix A4, Fig. A4.27,A4.28,A4.29). Similarly, no significant between-group differences were found for neck disability (*MD* (95% *CI*) = 6.94 points (−1.51, 15.39)) (Appendix A4, Fig. A4.30) or for the pain disability index (*MD* (95% *CI*) = 4.88 points (−3.52, 13.28)) (Appendix A4, Fig. A4.31). The certainty of the evidence for all outcomes was rated as very low according to the GRADE assessment (Appendix 3, Table A3).

A summary of the results for each comparison is presented in Table 3 (matrix of results).

Risk of bias assessment

The majority of studies (n = 12, 80%) were considered to be at high RoB,[13,28,29,31,32,43,45–48,50,51] whereas three (20%) studies were judged to have some concerns.[30,44,49] No study was classified as having a low RoB (Fig. 2). Among studies rated as having a high RoB or some concerns, the most common methodological limitations were related to deviations from intended intervention domains, missing outcome data, and outcome measurement (Fig. 3) (Appendix 5).

Certainty of the evidence

The certainty of the evidence for each outcome evaluated in this review is presented in Appendix 3. Overall, the certainty of the evidence was rated as very low, primarily because of small sample sizes, the high risk of bias and high heterogeneity of the studies. Considerable heterogeneity was observed regarding interventions, comparator treatments and outcome measures. Consequently, the data could not be pooled.

Discussion

This systematic review included 15 studies; however, synthesizing the findings was challenging due to substantial heterogeneity in the interventions evaluated, treatment dosage and intensity, comparator groups, and methods used to classify bruxism. Overall, the findings suggest that PT may reduce symptoms associated with bruxism, such as pain and muscle tenderness. Physiotherapy showed potential benefits compared with no treatment and demonstrated effects comparable to other conservative interventions, such as occlusal splint therapy. Combined approaches, particularly MT in conjunction with occlusal splints or kinesiotaping, showed the most promising results. However, the overall body of evidence was limited by methodological shortcomings and considerable clinical heterogeneity, including variations in interventions, diagnostic criteria and outcome measures. Consequently, firm conclusions regarding the effectiveness of PT cannot be drawn, underscoring the need for more standardized research in this field.

The 2025 International Consensus on Bruxism redefined the condition. However, most studies included in this review were conducted before publication of the updated consensus and therefore relied on outdated definitions. The findings of these studies were interpreted in light of the updated consensus. Five studies[28,43–46] assessed SB using tools consistent with the diagnostic criteria of the American Academy of Sleep Medicine, combining clinical evaluation with self-reported measures. Only 2 studies incorporated instrumental assessments (EMG, PSG), despite PSG remaining the gold standard for assessing SB. Moreover, the PSG protocols and diagnostic thresholds differed between the studies. Awake bruxism was assessed only via self-reports[29,30,32] often in combination with SB, whereas several older studies used poorly defined criteria.[13,31,47,48] These findings highlight the need for future studies to adopt standardized methods for assessing bruxism.

The findings of the present review are consistent with those of previous reviews,[2,4,27] which concluded that PT may benefit individuals with bruxism who experience associated symptoms such as pain or muscle tenderness, regardless of whether AB or SB is present.[2,4,27] However, the overall quality of the available evidence remains insufficient to support definitive clinical recommendations. Across the included studies, PT interventions were used to reduce pain, alleviate muscle tenderness, improve ROM, decrease excessive masticatory muscle activity, and ultimately improve quality of life. Although the available evidence suggests that these interventions may be beneficial, only a limited number of randomized and controlled clinical trials have been conducted, and most are characterized by a high risk of bias and substantial methodological limitations.

Physiotherapy may alleviate symptoms associated with bruxism through several complementary mechanisms. Manual therapy and myofascial release techniques target hyperactive masticatory muscles directly, reducing muscle tension, and trigger point sensitivity, thereby reducing pain and potentially decreasing excessive mechanical loading of the dentition. Stretching and guided exercises may improve muscle flexibility, coordination and jaw ROM, contributing to more efficient neuromuscular function.[52] Neuromodulatory interventions, including TENS and low-level laser therapy, may inhibit nociceptive signaling, modulate trigeminal motor reflexes, and decrease hyperexcitability of the jaw-closing muscles.[53] Furthermore, PT interventions incorporating relaxation training or biofeedback may reduce sympathetic nervous system activation, reducing stress-related parafunctional muscle activity, particularly

Table 3. Summary matrix of the results according to intervention, comparison, and outcome

Category	Information about the trials				Outcomes								
	therapy ONE	therapy TWO	bruxism symptoms	timepoint	bruxism activity	pain intensity	tenderness	mouth ROM	quality of life	sleep quality	stress level	trigger points	Fonseca Anamnestic Index
Manual therapy	manual therapy	home exercise	unspecified bruxism	final	☒[31]	☒[31]	?	?	☒[31]	❶[31]	☒[31]	❶[31]	❶[31]
	manual therapy	manual therapy + kinesiotaping	SB and AB associated with orofacial pain	final	?	☒[30]	☒[29,30]	?	?	?	?	?	?
	massage therapy	occlusal splint therapy	SB associated with TMD diagnosis	final	?	☒[43]	?	?	?	?	?	?	?
	massage therapy	occlusal splint therapy (silicon)	SB associated with TMD diagnosis	final	☒[49]	?	?	?	?	?	?	?	?
	massage therapy	massage therapy + occlusal splint therapy	SB associated with TMD diagnosis	final	?	❷[43]	?	?	?	?	?	?	?
	massage therapy	massage therapy + occlusal splint therapy	SB associated with TMD diagnosis	final	☒[49]	?	?	?	?	?	?	?	?
	massage therapy	no treatment	SB associated with TMD diagnosis	final	?	❶[43]	?	?	?	?	?	?	?
	massage therapy + occlusal splint therapy	occlusal splint therapy	SB associated with TMD diagnosis	final	?	❶[43]	?	?	?	?	?	?	?
	massage therapy + occlusal splint therapy	occlusal splint therapy	SB associated with TMD diagnosis	final	?	❶[49]	?	?	?	?	?	?	?
	massage therapy + occlusal splint therapy	no treatment	SB associated with TMD diagnosis	final	?	❶[43]	?	?	?	?	?	?	?
	massage therapy	no treatment	unspecified bruxism associated with TMD diagnosis and masticatory muscle pain	final	☒[51]	❶[51]	?	?	?	?	?	?	?
Stretching	muscles stretching + sleep hygiene advice	sleep hygiene advice (control group)	SB without any additional symptoms	final	☒[44]	?	❶[44]	☒[44]	?	?	?	?	?

Category	Information about the trials				Outcomes								
	therapy ONE	therapy TWO	bruxism symptoms	timepoint	bruxism activity	pain intensity	tenderness	mouth ROM	quality of life	sleep quality	stress level	trigger points	Fonseca Anamnestic Index
Active relaxation techniques	MyoMonitor training	biofeedback training	unspecified bruxism associated with tenderness in the masticatory muscles	final	❷[13]	?	?	?	?	?	?	?	?
	massed negative practice	occlusal splint therapy	SB without any additional symptoms	final	☒[45]	?	?	?	?	?	?	?	?
	massed negative practice	occlusal splint therapy	SB without any additional symptoms	six-month follow-up	☒[45]	?	?	?	?	?	?	?	?
	massed negative practice	strength exercise	SB without any additional symptoms	final	☒[45]	?	?	?	?	?	?	?	?
	massed negative practice	strength exercise	SB without any additional symptoms	one-month follow-up	☒[45]	?	?	?	?	?	?	?	?
	massed negative practice	biofeedback	SB without any additional symptoms	final	☒[45]	?	?	?	?	?	?	?	?
	massed negative practice	biofeedback	SB without any additional symptoms	one-month follow-up	☒[45]	?	?	?	?	?	?	?	?
	massed negative practice	education therapy	unspecified bruxism with any additional symptoms	final	☒[47]	?	?	?	?	?	?	?	?
	massed negative practice	no treatment	unspecified bruxism with any additional symptoms	final	☒[47]	?	?	?	?	?	?	?	?
Kinesiotaping	kinesiotaping	occlusal splint therapy	SB with any additional symptoms	final	?	☒[28]	☒[28]	☒[28]	?	?	?	?	?
TENS	TENS	MART	SB associated with symptoms of craniomandibular disorders	final	❷[46]	?	?	❷[46]	?	?	?	?	?
	TENS	Sham TENS	SB associated with symptoms of craniomandibular disorders	final	☒[46]	?	?	?	?	?	?	?	?
	TENS	occlusal splint therapy	unspecified bruxism associated with TMD diagnosis and myofascial pain	final	?	☒[50]	?	❷[50]	☒[50]	?	?	?	?
	TENS	occlusal splint therapy	unspecified bruxism associated with TMD diagnosis and myofascial pain	one-month follow-up	?	☒[50]	?	☒[50]	☒[50]	?	?	?	?
	TENS	MENS	unspecified bruxism associated with masticatory muscle pain	final	?	❷[48]	❷[48]	?	?	?	?	?	?
Multimodal treatment	multimodal treatment	waiting list	AB	final	?	❶[32]	?	❶[32]	?	?	?	?	?

❶ positive effect favoring therapy one; ❷ positive effect favoring therapy two; ? outcome not investigated; ☒ no significant difference between therapies. When a study reported multiple measures for the same outcome, the outcome was classified as positive if at least one measure showed a statistically significant benefit in favor of one of the interventions.

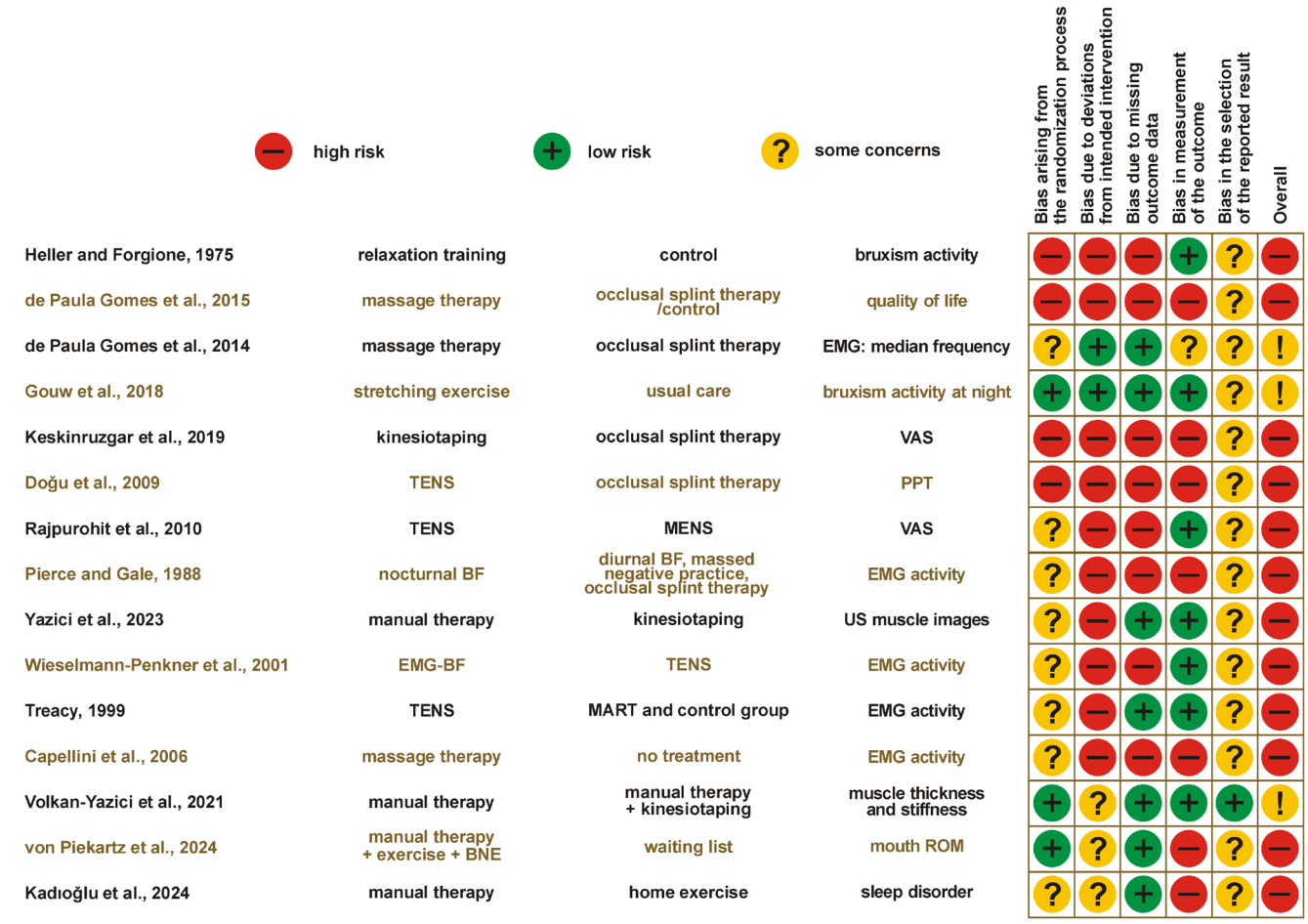


Fig. 2. Risk of bias assessment for each included study

EMG – electromyography; VAS – visual analogue scale; TENS – transcutaneous electrical nerve stimulation; PPT – pressure pain threshold; MENS – microcurrent electrical neuromuscular stimulation; BF – biofeedback; US – ultrasound; MART – muscular awareness relaxation training; ROM – range of motion.

in individuals with AB.[45,47] Collectively, these interventions target both peripheral musculoskeletal system and central neuromodulator mechanisms, supporting a multidimensional approach to managing bruxism and its adverse consequences.

With respect to bruxism activity, the available evidence suggests that improvements may be achieved with combined interventions, particularly MT combined with occlusal splint therapy and MART. Similarly, massage combined with occlusal splint therapy resulted in greater reductions in pain intensity than massage alone. The authors propose that combining these 2 approaches could enhance local blood flow and nutrient delivery, thereby improving muscle activity (i.e., normalizing muscle function) and reducing pain. Doğu et al. reported that occlusal splint therapy was more effective than TENS in reducing tenderness of the masticatory muscles.[50] This difference may reflect the distinct mechanisms of action of the 2 interventions. Transcutaneous electrical nerve stimulation primarily provides temporary analgesia through neuromodulatory mechanisms, acting predominantly on pain modulation without addressing the primary cause.[53] In contrast, occlusal splints act mechanically by stabilizing the relationship between the arches and reducing excessive loading of masticatory muscles associated with bruxism.[43] These results are consistent with previous concepts related to occlusal splint treatment, which emphasize its role in bruxism symptom relief. Occlusal splints are reversible and non-invasive and may provide sustained symptom relief when used consistently. However, their effectiveness depends largely on patient adherence to the prescribed treatment regimen.

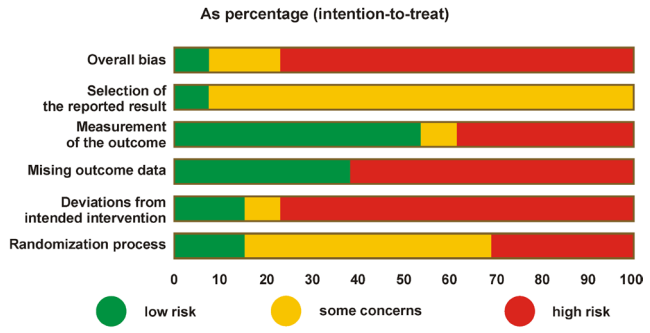


Fig. 3. Overall risk of bias across the included studies

Regarding jaw ROM, no significant differences between groups were observed in most comparisons. Neither stretching nor kinesiotaping demonstrated clinically meaningful improvements in active, pain-free, or passive ROM.[44] It is important to note, however, that restricted jaw ROM is rarely reported in individuals with bruxism.

An additional consideration is the coexistence of bruxism with other clinical conditions. Three studies included participants with bruxism and associated TMD, making it difficult to determine whether the observed changes in outcomes were due to TMD symptoms, bruxism, or both. Symptoms such as pain and limited ROM are more prevalent in individuals with TMD than in those with bruxism alone. Consequently, findings from studies involving participants with both conditions cannot be directly compared with studies that included individuals with bruxism only.

Risk of bias and certainty of evidence

As previously mentioned, the RoB varied across the included studies. Most studies were classified as having a high risk of bias, several raised some concerns, and none were judged to have a low risk of bias. The most common methodological limitations were inadequate reporting of the randomization process and insufficient information regarding the blinding of participants, therapists, and outcome assessors. Blinding is particularly challenging in PT trials because interventions are typically delivered directly by therapists or performed by patients, increasing the potential for performance bias. Among the studies included in this review, only 2 described their blinding procedures,[43,49] 1 study had no blinding,[44] and the remaining 12 provided no information of blinding, indicating a substantial risk of bias in this domain.

Inadequate randomization and allocation concealment may substantially influence treatment effects. When participant allocation is non-random or predictable, selection bias may occur, whereby patients with characteristics such as greater motivation or less severe symptoms are preferentially allocated to the intervention group. Similarly, inadequate allocation concealment may allow therapists or investigators to influence group assignment, resulting in systematic differences between groups at baseline. These methodological flaws may exaggerate the apparent effectiveness of PT, particularly for subjective outcomes such as self-reported pain and muscle tenderness, which are susceptible to expectation and placebo effects. For instance, in studies with poorly implemented allocation procedures, improvements reported in the intervention group could partially reflect baseline differences rather than a true therapeutic effect. Additionally, poorly executed randomization and allocation increase the risk of selection, performance and attrition bias.[39–41]

Additional shortcomings further contribute to attrition bias. Most studies provided limited information regarding study limitations, participant adherence, or reasons for missing outcome data. Failure to account appropriately for missing data may lead to biased or overestimated treatment effects.[39] Furthermore, the long-term effectiveness of PT remains unclear, as only 1 study included long-term follow-up, whereas the duration of treatment was generally short, with the exception of the study by Treacy,[46] which lasted 4 months. Collectively, these limitations warrant cautious interpretation of the reported effectiveness of PT for individuals with bruxism.

The certainty of the evidence, assessed using the GRADE approach, was predominantly rated as very low due to methodological limitations and high heterogeneity among the studies. The majority of studies included a small number of participants, further limiting the strength of the evidence.

Clinical implications

This review provides a comprehensive overview of PT interventions that have been investigated for the management of bruxism with the aim of determining the most effective intervention. Although the available evidence does not allow definitive recommendations regarding the most effective intervention, several approaches appear promising for improving pain, muscle tenderness, and, when impaired, maximal mouth opening immediately after treatment. Overall, PT can be a good alternative strategy for improving pain, MMO, and tenderness (when present) immediately after treatment, especially when carried out in an interdisciplinary way. Manual therapy, active relaxation techniques and multimodal PT programs could be considered the first treatment option to manage the consequences of bruxism. Importantly, in the included studies, PT was used to manage the adverse consequences of bruxism, and not the bruxism behavior.

Research implications

The optimal PT approach for individuals with bruxism remains unclear due to methodological weaknesses, small sample sizes, short intervention periods, and inconsistent methods for diagnosing and classifying bruxism. Future trials should adopt standardized, consensus-based diagnostic criteria and validated assessment tools for both AB and SB to improve the comparability of study findings. Methodological quality should be strengthened through robust randomization procedures, appropriate allocation concealment, comprehensive reporting of blinding, participant adherence, and study limitations. In addition, strategies for handling missing data should be clearly described and based on appropriate statistical methods to minimize the risk of bias.

Future research should also establish adequate treatment durations and include sufficiently long follow-up periods to exert measurable effects. This will ensure reporting of long-term outcomes to evaluate therapeutic

benefits over a longer period. Larger, adequately powered clinical trials are needed, following recommendations by Zielinski et al.[54] regarding minimum sample sizes for PT research. Reporting standardized effect sizes and measures of clinical importance would further improve the interpretation and clinical applicability of future findings. Additionally, comparative trials examining different types of bruxism within the same study design would further elucidate potential variations in intervention outcomes.

The evidence synthesized in this review largely reflects earlier concepts of bruxism, which emphasized its adverse musculoskeletal consequences, including pain and dysfunction. As knowledge of bruxism continues to evolve, specialized orofacial physiotherapists increasingly recognize its multifactorial nature and complexity. Therefore, future interventions may integrate musculoskeletal treatment with neuromuscular, behavioral and psychological strategies.[55] These methods may combine bruxism neuroscience education (BNE), motor control training, and parafunctional behavior-specific exercises tailored to each patient.[32] Such multidimensional approaches may influence future research outcomes. Finally, it should be acknowledged that most studies included in this review were published more than 5 years ago. Given the considerable advances in both bruxism research and PT practice since then, further high-quality studies based on current conceptual frameworks are needed.

Strengths and limitations

A major strength of this systematic review is its comprehensive search strategy, which was developed in collaboration with an experienced librarian and conducted without language or publication date restrictions. The broad PICO-based eligibility criteria enabled the inclusion of a wide range of PT approaches for bruxism. However, this broad scope also resulted in significant heterogeneity, limiting comparisons across studies and precluding quantitative synthesis. In addition, several studies inadequately reported numerical outcome data, preventing the calculation of effect sizes or independent verification of the reported findings. Although attempts were made to contact study authors to obtain missing information, this was not always possible, particularly for older publications, further impacting data verification and consistency.

Although broad inclusion criteria were used, only RCTs and CTs were included in this review. Although this decision reduced the number of eligible studies, these designs provide the highest level of evidence for evaluating treatment effectiveness. Additionally, most studies did not specify whether participants with bruxism were included or excluded, partly because of evolving definitions. Only one study focused exclusively on AB, whereas the remaining studies investigated SB, mixed populations, or unspecified bruxism. The present review adopted the most recent consensus definitions,[9,12] highlighting the importance of distinguishing between bruxism types. Future studies should clearly define and report bruxism subtypes, staying consistent to the updated guidelines and enhancing the consistency and comparability of research findings.

Finally, the considerable heterogeneity in intervention type, treatment intensity, duration, comparator groups, and outcome measures prevented the formulation of specific clinical recommendations. Accordingly, the findings of this review should be interpreted as a summary of the currently available evidence regarding PT interventions for managing the adverse consequences of bruxism rather than as definitive recommendations for clinical practice.

Conclusions

Physiotherapy for the management of bruxism remains an emerging area of research, with important gaps in the current evidence. Existing studies have primarily focused on reducing the adverse consequences of bruxism in symptomatic individuals. Methodological limitations, heterogeneous interventions, and evolving definitions of bruxism continue to limit the interpretation and generalizability of the available evidence. Recent advances in the understanding and assessment of bruxism have enabled a clearer distinction between the mechanisms underlying awake and sleep bruxism and have emphasized that currently available assessment tools measure different aspects of the behavior rather than the behavior itself. These developments provide an opportunity for future research to adopt more standardized, precise, and clinically meaningful methodologies. Given that bruxism is currently understood as a motor behavior, physiotherapists, with their expertise in the understanding and modulation of movement are well positioned to contribute to its management. When integrated within an interdisciplinary framework, PT interventions may help alleviate the musculoskeletal and neuromodulatory consequences of bruxism, offering a holistic approach to mitigating symptoms and improving patient outcomes. Future research should focus on rigorous, well-designed trials to establish evidence-based PT protocols that are both effective and sustainable.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets generated and/or analyzed during the current study, including the Appendix, 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 relationship between sleep bruxism and saliva: A systematic review and meta-analysis

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Abstract

Sleep bruxism (SB) is defined as rhythmic masticatory muscle activity occurring during sleep. Evidence suggests that SB may be associated with alterations in salivary function; however, findings remain inconsistent across age groups. This study aimed to evaluate the relationship between SB and salivary flow and composition. A systematic search of PubMed®, Embase® and Web of Science was conducted to identify human studies reporting SB and saliva-related parameters. Twenty studies involving 4,543 participants were included. Risk of bias was assessed using the Risk of Bias Assessment tool for Non-randomized Studies (RoBANS-2), and certainty of evidence was appraised using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) framework. In pediatric populations, SB was frequently associated with sleep drooling, with a pooled prevalence of 77.4% and a significant association between SB and drooling (odds ratio (*OR*): 2.88, 95% confidence interval (*CI*): 1.53–5.42; $I^2 = 91\%$). In adults, 2 studies reported inconsistent findings regarding objectively measured salivary flow, and 1 study found an association with subjective xerostomia. A meta-analysis of 4 studies indicated elevated salivary cortisol in participants with SB (standardized mean difference (*SMD*): 0.86, 95% *CI*: 0.44–1.29), although studies using polysomnography for SB diagnosis found no significant differences. Results for chromogranin A (CgA) and α-amylase were heterogeneous. Overall, the available evidence, which was of very low certainty, suggests that SB is associated with hypersalivation in pediatric populations and with subjective oral dryness in adults, whereas associations with salivary biomarkers remain inconclusive. Future studies using standardized, age-specific protocols that combine objective SB diagnostic methods with controlled saliva sampling are warranted to clarify the underlying mechanisms and inform clinical management.

Keywords: saliva, sleep bruxism, age groups, salivary proteins and peptides, sialorrhea

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Highlights

- This review identifies age-dependent differences in the association between sleep bruxism (SB) and salivary outcomes in children, adolescents and adults.
- Sleep bruxism shows a consistent link with hypersalivation and drooling in children and adolescents, with implications for orthodontic appliance design and patient comfort.
- In adults, SB is associated with perceived oral dryness and variable salivary flow, influenced by stress and comorbid sleep disorders.
- The findings support age-specific assessment protocols using objective SB diagnosis, standardized saliva sampling and control of confounding factors.

Introduction

Sleep bruxism (SB), with a global prevalence of approx. 21%, is a common masticatory muscle activity occurring during sleep that is characterized as rhythmic (phasic) or non-rhythmic (tonic).[1,2] Its etiology is multifactorial, involving genetic, neurochemical and sleep arousal-related mechanisms, as well as lifestyle factors such as smoking, caffeine consumption and alcohol intake.[3–6] The condition has also been associated with headaches, oxidative stress and other comorbidities, including obstructive sleep apnea (OSA) and gastroesophageal reflux disease.[7–11] Sleep bruxism is currently regarded as a sleep-related behavior rather than a movement or sleep disorder.[1] Although SB is generally not harmful, it may increase the risk of dental wear, orofacial pain, temporomandibular disorders, and prosthodontic complications, such as crown fracture and implant overload.[12–14]

Beyond its mechanical consequences, emerging evidence suggests that SB may influence salivary secretion, which is essential for maintaining oral health and supporting the long-term success of dental restorations.[15–17] Saliva protects oral tissues through lubrication, antimicrobial activity and buffering, and is particularly important in oral rehabilitation settings.[18] Several studies have suggested that SB may increase salivary flow as a protective response to airway obstruction in OSA or acid exposure in gastroesophageal reflux disease.[15,19,20] These findings have further supported the redefinition of SB as a behavior rather than a disorder.[1,21] Conversely, other studies have reported reduced salivary secretion in adults with SB, potentially due to chronic stress and autonomic dysregulation.[22,23] The inconsistent evidence may result from methodological differences, age-related variations and the influence of comorbid conditions such as OSA and xerostomia.[24–26] Additionally, stress-related salivary biomarkers, including cortisol, chromogranin A (CgA) and α-amylase have also been investigated in individuals with SB, but their clinical relevance remains unclear.[27–32] Importantly, objective salivary hypofunction (measured flow reduction) should be distinguished from subjective xerostomia (perceived dryness despite normal flow) as both have been reported in SB studies.[33–36]

Although previous studies have examined some aspects of the relationship between SB and salivary function, no systematic review has comprehensively synthesized the available evidence across children, adolescents and adults. Addressing this gap is clinically relevant due to the high prevalence of SB worldwide[2] and its reported associations with other comorbidities.[7–11] Clarifying whether SB is consistently linked to altered salivary secretion or stress-related biomarkers may also inform clinical practice in pediatric dentistry, orthodontics, prosthodontic rehabilitation, and sleep medicine.

Therefore, this systematic review and meta-analysis aimed to: 1) evaluate the association between SB and salivary flow across different age groups; 2) assess the relationship between SB and salivary components; and 3) explore potential mechanisms linking SB with altered salivary secretion.

Material and methods

Study design

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines.[37] The review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) (registration No. CRD42024582843). The protocol and reporting approach were designed to ensure methodological transparency and reproducibility.

Search strategy

A systematic search was conducted in PubMed®, Embase® and Web of Science to identify relevant studies published up to August 15, 2024. The search strategy combined keywords related to SB and salivary secretion. In PubMed® and Embase®, the index terms (Medical Subject

Headings (MeSH) and Emtree terms, respectively) were further added to the search strategy. The search was limited to articles published in English (Supplementary Material A (available on request from the corresponding author)). Reference lists of eligible studies were manually screened to identify additional relevant publications.

Article screening and eligibility criteria

Study selection was performed according to the Population, Exposure, Outcome (PEO) framework (Table 1).[38]

Article screening was conducted in 2 stages: title and abstract screening, followed by full-text review, by 2 reviewers (DL and HC). Discrepancies were resolved by consensus or consultation with a senior reviewer (FW).

The inclusion criteria were: 1) human studies; 2) studies assessing both SB and saliva-related outcomes using questionnaires, clinical inspection or objective measurements; 3) observational studies, controlled clinical trials and randomized controlled clinical trials; 4) intervention studies providing sufficient baseline data to analyze the association between SB and salivary secretion.

Case reports, case series, reviews, editorials, commentaries, conference abstracts, non-English publications, animal studies, and studies without full-text availability were excluded.

Quality assessment

The quality of the included studies was assessed using a revised version of the Risk of Bias Assessment tool for Non-randomized Studies (RoBANS-2) (Supplementary Material B).[39] The RoBANS-2 evaluates 8 domains: comparability of the target group; target group selection; confounders; measurement of intervention/exposure; blinding of assessors; outcome assessment; incomplete outcome data; and selective outcome reporting. The risk of bias for each domain was rated as high, low or unclear.

Certainty of evidence was appraised using the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) framework,[40] considering risk of bias, inconsistency, indirectness, imprecision, and publication bias. Because this review did not formulate clinical recommendations, only the certainty of evidence component of the GRADE framework was applied.

Table 1. Population, exposure, outcome (PEO) framework

Element	Standard
Population	children, adolescents and adults with SB
Exposure	presence of sleep bruxism, diagnosed using self-report, clinical examination or instrumental methods (e.g., PSG, EMG)
Outcome	salivary outcomes, including salivary flow rate, hypersalivation (sleep drooling), subjective xerostomia, and stress-related salivary biomarkers (cortisol, CgA, α-amylase)

SB – sleep bruxism; PSG – polysomnography; EMG – electromyography; CgA – chromogranin A.

Publication bias was evaluated using funnel plots and Egger's regression test when at least 10 studies were available for an outcome.

Two reviewers (DL and HC) independently performed the quality assessment of the included studies. Disagreements were resolved through discussion, or, when necessary, consultation with a senior reviewer (FW).

Data extraction

Data was independently extracted by 2 reviewers using a standardized form and cross-checked for accuracy. The extracted data included authors, publication year, study location, study design, sample characteristics (sample size, age and sex distribution); SB assessment methods (e.g., self-report, clinical exam, polysomnography (PSG)); saliva assessment methods (e.g., salivary flow measurement, biomarker analysis); and the main findings regarding the association between SB and salivary secretion. When subgroups were present, their age, sex and sample characteristics was presented separately. Data extraction was performed independently by 2 reviewers (DL and HC).

Meta-analysis

The quantitative synthesis evaluating the relationship between SB and salivary secretion was performed in the R language (v. 4.4.1; R Foundation for Statistical Computing, Vienna, Austria) with the R packages "meta"[41] and "metafor"(v. 4.6-0).[42] The number of individuals with drooling or hyposalivation in the SB and control groups was used to calculate odds ratios (*OR*s) and 95% confidence intervals (*CI*s). For continuous outcomes, such as salivary cortisol concentrations, mean differences (*MD*s) and the standardized mean differences (*SMD*s) were calculated. A random effects model was used, and heterogeneity was assessed using the I^2 index. Thresholds for the interpretation of the I^2 statistic followed the Cochrane Handbook[43]: 0–40% – heterogeneity might not be important; 30–60% – may represent moderate heterogeneity; 50–90% – may represent substantial heterogeneity; 75–100% – considerable heterogeneity.

Results

Study selection

The initial search yielded a total of 325 articles, including 49 from PubMed®, 191 from Embase® and 84 from Web of Science. After removing duplicates, 230 articles remained. An additional article was identified through manual searching, resulting in 231 articles for the title and abstract screening. Following this, 37 articles were selected for full-text review. Of these, 20 studies met the inclusion

criteria and were included in the qualitative synthesis; 2 outcomes (sleep drooling in children and salivary cortisol) were eligible for meta-analysis. The study selection process is presented in Fig. 1.

Study characteristics

Twenty studies involving a total of 4,543 participants were included. The studies showed a broad geographical distribution, with 1 from North America (USA), 7 from South America (Brazil), 2 from the Middle East (Iran), 4 from Europe (2 from Greece, 2 from Serbia), and 6 from Asia (5 from Japan and 1 from Romania). Nine studies[44–52] focused on children and adolescents, 10 studies[28–30,34–36,53–56] on adults, and one,[31] specifically, on older adults. The characteristics of the included studies are summarized in Table 2.

All included studies were observational, comprising 17 cross-sectional studies,[28–31,34,35,44–51,53,54,56] 2 cohort studies[36,55] and 1 case–control study.[52] In addition, 5 studies[30,36,52,55,56] compared individuals with and without SB.

The included studies used various instrumental and non-instrumental methods to assess SB. Specifically, 5 studies relied solely on self-report questionnaires,[34,35,48,50,52] 10 studies used clinical examinations with or without additional questionnaires,[30,31,36,44–47,49,53,55] and 5 employed instrumental approaches such as PSG, electromyography (EMG) or BiteStrip devices.[28,29,51,54,56] Among them, 2 studies[54,56] used PSG for SB assessment.

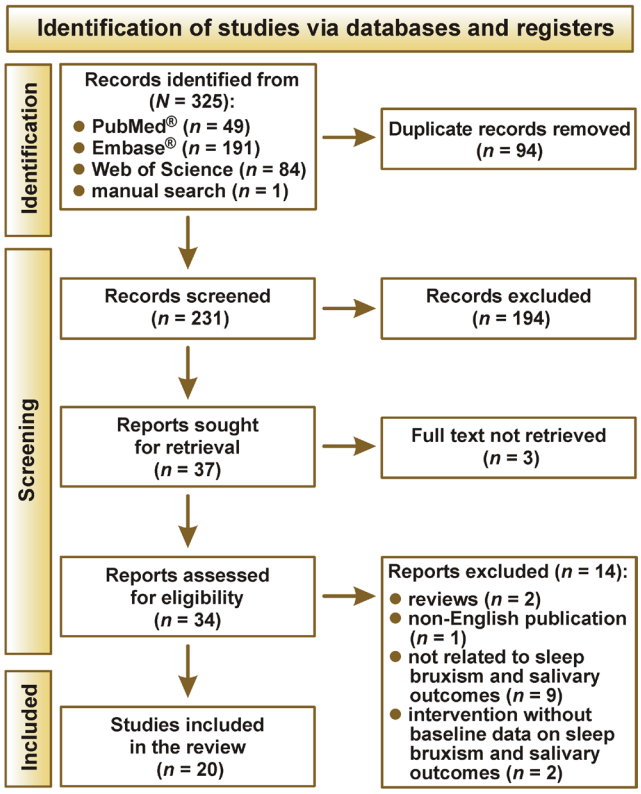


Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 flow diagram of the study selection process

In terms of salivary assessment, studies utilized subjective and objective approaches. Seven studies adopted questionnaires[35,44,48–52] to collect information on drooling or dry mouth. For objective assessment, salivary flow rate was analyzed using the spitting method or the Schirmer test in 2 studies.[35,55] Stress-related salivary biomarkers were also evaluated, including salivary cortisol in 8 studies,[28,30,34,36,46,47,54,56] CgA in 3 studies[29,53,54] and alpha-amylase in 2 studies.[28,31]

Risk of bias of the included studies

The results of the risk-of-bias assessment are presented in Table 3. Most studies showed a low risk of bias for target group comparability (19 studies[28–31,35,36,44–56]) and adequate adjustment for confounders (18 studies[28–31,34,36,44–49,51–56]). Seven studies[29,35,36,45,50,52,54] had a high risk of bias due to selection of the target group from specific settings, such as clinics, military personnel or dental setting, which limited the generalizability of the findings.

Regarding outcome assessment, 6 studies assessing SB[34,36,44,48,50,52] and 7 studies evaluating salivary outcomes[44,45,48,49,50–52] used only questionnaires and were therefore considered to have a high risk of bias. Blinding of the assessors was not clearly reported in any of the 20 studies and was therefore rated as unclear. One study[45] had a high risk of bias due to incomplete outcome data, whereas none of the included studies showed evidence of selective outcome reporting.

Sleep bruxism and salivary flow rate

Seven studies[44,45,48–52] investigated the relationship between SB and salivary flow rate, mainly sleep drooling, in children and adolescents. All these studies employed questionnaires or interviews to assess both SB and salivary secretion outcomes, and 4 studies[44,45,49,55] additionally incorporated clinical inspection of tooth wear or other symptoms as part of the SB assessment. Four of the 7 studies[48,50–52] reported the prevalence of drooling in children with SB, ranging from 9% to 82%. When pooled, the overall prevalence of drooling among children with SB was 77.4%, which is approximately twice that observed in children without SB (37.5%). In addition, 5 studies[44,48,50–52] reported a positive association between SB and drooling, whereas the remaining 2 studies[45,49] found no significant correlation. A meta-analysis of 5 studies[48–52] with available data showed that children with SB are significantly more likely to experience drooling during sleep (*OR* = 2.88, 95% *CI*: 1.53–5.42) (Fig. 2). Notably, the heterogeneity was substantial (I^2 = 91%). However, all 5 contributing studies assessed both SB and drooling using self- or parent-report questionnaires, none used instrumental or standardized clinical measures, and age strata were inconsistently reported. Accordingly, formal subgroup or sensitivity analyses were not feasible.

Two studies[35,55] investigated the relationship between SB and salivary flow in adults. One study[55] reported a slightly lower, although not statistically significant, stimulated salivary flow rate, measured using the spitting method, in adults with SB compared with controls. The other study,[35] conducted among adult male soldiers, found that SB was not associated with unstimulated morning hyposalivation, as measured by the Schirmer test, but was significantly associated with the subjective sensation of dry mouth.

Sleep bruxism and salivary components

Eleven studies investigated the association between SB and salivary components, mainly stress-related salivary biomarkers, specifically cortisol (n = 8),[28,30,34,36,46,47,54,56] CgA (n = 3)[29,53,54] and alpha-amylase (n = 2).[28,31] Nine studies reported alterations in salivary biomarkers in individuals with SB compared with controls. Specifically, 6 studies[28,30,34,36,46,53] found significant positive associations between SB and stress-related biomarkers, 3 studies[29,31,47] reported negative correlations, and 2 studies[54,56] displayed no correlation.

Among the studies investigating cortisol, 2 were conducted in children. One study involving 100 children (mean age = 7.2 years) reported significantly lower salivary cortisol levels in those with SB,[47] whereas the other, conducted in a larger pediatric cohort of 551 children aged 7–8 years, found higher cortisol levels in individuals with SB.[46]

Four studies[28,30,34,56] investigating the association between SB and salivary cortisol levels in adults were eligible for meta-analysis. The pooled analysis showed higher salivary cortisol levels in individuals with SB than in controls (*SMD*: 0.86, 95% *CI*: 0.44–1.29) (Fig. 3). Moderate-to-substantial heterogeneity was observed (I^2 = 59%). The included studies used different methods for SB assessment (Table 2), and saliva sampling protocols and analytical assays also varied. Because a subgroup analysis based on the diagnostic method would have resulted in the PSG subgroup containing only a single study, a formal subgroup meta-analysis was not performed. Notably, the 2 studies[54,56] using PSG to evaluate SB in adults consistently reported that salivary cortisol levels were not associated with SB severity and did not differ significantly between bruxers and non-bruxers.

For CgA and α-amylase, all relevant studies were conducted in adults and reported mixed findings. Two studies[53,54] found a positive association between SB and CgA, whereas another found a negative correlation.[29] Regarding α-amylase, 1 study[31] among older adults (>65 years) reported a negative association with SB, whereas another study[28] among participants aged 25–55 years found no significant differences between bruxers and non-bruxers.

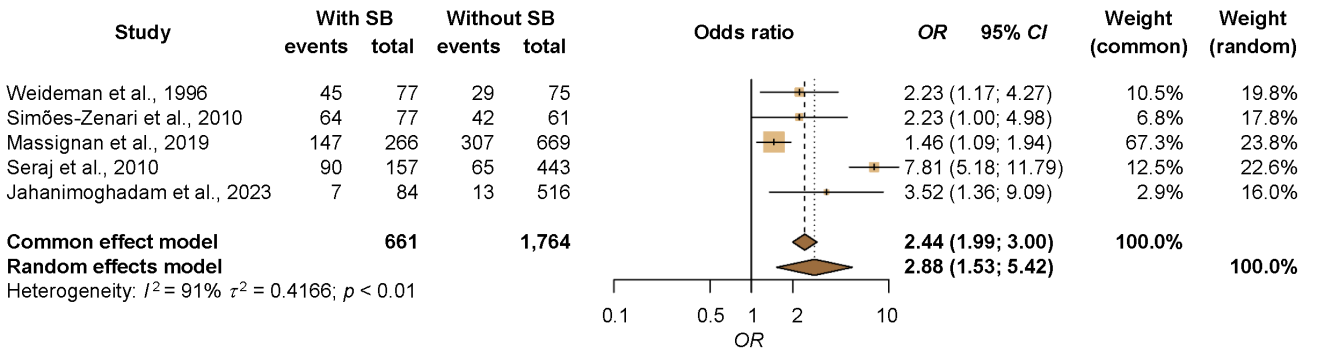


Fig. 2. Forest plot showing the association between sleep bruxism (SB) and sleep drooling

OR – odds ratio; *CI* – confidence interval.

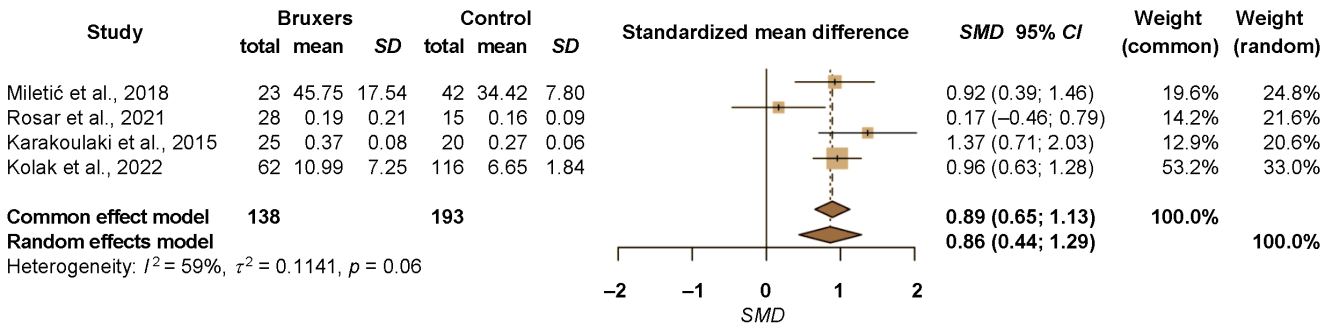


Fig. 3. Forest plot showing the standardized mean difference (*SMD*) in salivary cortisol levels between individuals with and without sleep bruxism (SB)

Table 2. Characteristics of the included studies

Study	Country	Sample size, *n*	Age [years]	Sex	Study type
Weideman et al. 1996[52]	USA	152 (77 patients with SB, 75 controls)	2–18	patients with SB: 38M, 39F controls: 41M, 34F	case–control
Miyawaki et al. 2004[55]	Japan	16	patients with SB: 29.8 ±6.6 controls: 28.9 ±6.0	patients with SB: 7M, 1F controls: 6M, 2F	cohort
Makino et al. 2009[29]	Japan	46	21–45 (mean = 23.9)	23M, 23F	cross-sectional
Simões-Zenari et al. 2010[51]	Brazil	141	4–6	71M, 70F	cross-sectional
Seraj et al. 2010[50]	Iran	600	4–12 (7.4 ±2.4)	314M, 286F	cross-sectional
Abekura et al. 2011[53]	Japan	76	males: 23.3 females: 24.2	36M, 40F	cross-sectional
Castelo et al. 2012[47]	Brazil	100	7.2 ±0.6	NA	cross-sectional
Karakoulaki et al. 2015[28]	Greece	45	25–55	20M, 25F	cross-sectional
Miletić et al. 2018[30]	Serbia	65 (23 patients with SB, 42 controls)	patients with SB: 20–34 controls: 20–35	patients with SB: 4M, 19F controls: 13M, 29F	cross-sectional
Massignan et al. 2019[49]	Brazil	935 children (372 with primary dentition, 563 with mixed dentition)	primary dentition: 3.6 ±1.0 mixed dentition: 9 ±0.8	408M, 527F	cross-sectional
de Lima Bach et al. 2019[46]	Brazil	551	7–8	291M, 260F	cross-sectional
Haraki et al. 2019[54]	Japan	54	23.8 ±2.1	NA	cross-sectional
Morita et al. 2020[31]	Japan	219	>65	77M, 142F	cross-sectional
Apessos et al. 2020[35]	Greece	173 male soldiers	18–30 (23.1 ±2.9)	M only	cross-sectional
Fluerașu et al. 2021[36]	Romania	60 healthy students	23.1 ±2.04 patients with SB: 24 (*Me*) controls: 23 (*Me*)	patients with SB: 12M, 18F controls: 15M, 15F	cohort
Rosar et al. 2021[56]	Brazil	43 (28 patients with SB, 15 controls)	19–30 patients with SB: 22.5 ±2.7 controls: 21.6 ±1.7	NA	cross-sectional
Kolak et al. 2022[34]	Serbia	178 students	19–30	84M, 94F	cross-sectional
Jahanimoghadam et al. 2023[48]	Iran	600 schoolchildren	6–12 (9.1 ±1.8)	327M, 273F	cross-sectional
de Alencar et al. 2017[45]	Brazil	66 children (34 patients with SB, 32 controls)	3–7 (5.3 ±1.1)	27M, 39F	cross-sectional
Aguiar et al. 2024[44]	Brazil	403 adolescents	12–19 (14.4 ±1.6)	169M, 234F	cross-sectional

Me – median; NA – not available; M – male; F – female; QNR – questionnaire; CI – clinical inspection; AASM – American Academy of Sleep Medicine; ELISA – enzyme-linked immunosorbent assay; EIA – enzyme immunoassay.

Bruxism diagnosis/ measurement	Salivary diagnosis/ measurement	Main results
QNR (grinding sound)	QNR	Patients with SB had approximately a twofold higher likelihood of drooling during sleep than controls.
QNR + CI (tooth wear, jaw muscle fatigue)	salivary flow rate (spitting method)	Patients with SB tended to have a slightly lower salivary flow rate than controls, but the difference was not statistically significant.
QNR, study casts, BiteStrip	CgA level (EIA kit and protein assay kit)	Daytime psychological stress level was significantly negatively correlated with SB behavior.
QNR, orofacial motricity assessment	QNR	Sialorrhea was identified as an associated factor, and 67% of children with drooling exhibited SB.
QNR	QNR	A significant association was observed between sleep drooling and SB ($p < 0.001$). In children with drooling, the prevalence of SB (58%) was twice that observed in children without drooling.
QNR, CI (AASM)	CgA level (ELISA kit and protein assay kit)	The mean salivary CgA concentration was significantly increased in the SB group following a stress task.
QNR, CI	cortisol level (ELISA)	Children with SB had significantly lower awakening salivary cortisol concentrations than controls.
QNR, BiteStrip	cortisol level (ELISA), α-amylase level (enzyme kinetic assay)	Patients with SB showed higher salivary cortisol levels than non-bruxers ($p < 0.001$). Salivary α-amylase levels did not differ significantly between individuals with and without SB ($p = 0.414$).
QNR, CI	cortisol level (chemiluminescence)	Patients with SB had higher levels of salivary cortisol than controls.
QNR, CI (tooth wear)	QNR	Drooling and tooth wear were not associated with probable SB in either the primary or mixed dentition.
QNR, CI (AASM)	cortisol level (electrochemiluminescence)	Children with SB had higher salivary cortisol levels and greater difficulty maintaining biological rhythm than children without SB.
PSG	cortisol and CgA levels (EIA)	There were no significant differences in sleep architecture, psychological status or salivary cortisol levels among groups with different frequencies of SB episodes. Morning salivary CgA levels were slightly lower in the low-frequency SB group than in the high-frequency SB group.
CI	α-amylase level (salivary α-amylase monitor)	Sleep bruxism was significantly negatively correlated with salivary α-amylase levels ($p < 0.05$).
QNR	QNR, salivary flow rate (Schirmer test)	Morning hyposalivation was associated with poor sleep quality but not with excessive daytime sleepiness or SB. A significant association was also observed between subjective feeling of dry mouth and poor sleep quality, excessive daytime sleepiness, an increased risk of obstructive sleep apnea, and SB.
QNR	cortisol level (ELISA)	Subjects with SB had higher levels of general anxiety and stress. Salivary cortisol levels were also higher in subjects with SB and were correlated with general anxiety, but not with work-related psychological state.
QNR, CI, PSG	cortisol level (ELISA)	Salivary cortisol levels were not associated with SB severity and did not differ significantly between bruxers and non-bruxers.
QNR	cortisol level (ELISA)	Probable SB was significantly associated with higher stress levels and higher mean salivary cortisol concentrations.
QNR	QNR	Sleep bruxism was significantly associated with age, gastrointestinal disease, drooling, mouth breathing, nasal obstruction, oral habits, sleep disorders, jaw disorders, and family history of bruxism.
QNR, CI (AASM)	interview	Drooling, sleep-talking, nocturnal awakenings, sleepwalking, and nocturnal enuresis were not associated with SB in the final model.
QNR, CI	QNR	Drooling on the pillow was significantly associated with SB ($\beta = 0.184$, $p < 0.05$).

Table 3. Quality assessment of the studies included in the review using the Risk of Bias Assessment tool for Non-randomized Studies (RoBANS-2)

Study	Risk of bias							
	comparability of the target group	target group selection	confounders	measurement of exposure	blinding of assessors	outcome assessment	incomplete outcome data	selective outcome reporting
Weideman et al. 1996[52]	low	high	low	high	unclear	high	low	low
Simões-Zenari et al. 2010[51]	low	low	low	high	unclear	high	low	low
Seraj et al. 2010[50]	low	high	high	high	unclear	high	low	low
Massignan et al. 2019[49]	low	low	low	low	unclear	high	low	low
Apessos et al. 2020[35]	low	high	high	low	unclear	low	low	low
Kolak et al. 2022[34]	high	low	low	low	unclear	low	low	low
Jahanimoghadam et al. 2023[48]	low	low	low	high	unclear	high	low	low
de Alencar et al. 2017[45]	low	high	low	low	unclear	high	high	low
Haraki et al. 2019[54]	low	high	low	low	unclear	low	low	low
Rosar et al. 2021[56]	low	low	low	low	unclear	low	low	low
de Lima Bach et al. 2019[46]	low	low	low	low	unclear	low	low	low
Miletić et al. 2018[30]	low	low	low	low	unclear	low	low	low
Miyawaki et al. 2004[55]	low	low	low	low	unclear	low	low	low
Morita et al. 2020[31]	low	low	low	high	unclear	low	low	low
Makino et al. 2009[29]	low	high	low	low	unclear	low	low	low
Abekura et al. 2011[53]	low	low	low	low	unclear	low	low	low
Castelo et al. 2012[47]	low	low	low	low	unclear	low	low	low
Karakoulaki et al. 2015[28]	low	low	low	low	unclear	low	low	low
Fluerașu et al. 2021[36]	low	high	low	high	unclear	low	low	low
Aguiar et al. 2024[44]	low	low	low	low	unclear	high	low	low

Certainty of evidence (GRADE)

The certainty of evidence for the main outcomes was assessed using the GRADE framework.[40] As all included studies were observational, the initial certainty was rated as low and was further downgraded because of risk of bias, inconsistency and imprecision. Publication bias could not be assessed because neither meta-analysis included 10 or more studies. Overall, the certainty of evidence was rated as very low for all outcomes, including salivary flow rate (drooling) in children and adolescents, salivary flow rate in adults, and stress-related salivary biomarkers (cortisol, CgA and α-amylase) (Table 4).

Publication bias

As both meta-analyses in this review included fewer than 10 studies, formal assessment of publication bias was not performed, in accordance with the recommendation of the Cochrane Handbook and the GRADE handbook.[40,43]

Table 4. Grading of Recommendations, Assessment, Development and Evaluation (GRADE) summary of findings for the association between sleep bruxism (SB) and salivary outcomes

Outcome	Relative effect/study findings	Participants, *n*	Quality of evidence (GRADE)	Comments
Salivary flow rate in children and adolescents (drooling)	*OR*: 2.88 (95% *CI*: 1.60–5.17), $p < 0.001$	2,448 (5 studies)	⊕◯◯◯ very low	high heterogeneity ($I^2 = 91\%$); mostly self-reported
Salivary flow rate in adults	mixed results: 1 study reported a slightly reduced salivary flow rate, whereas another found no objective difference but a higher prevalence of subjective xerostomia	189 (2 studies)	⊕◯◯◯ very low	very small samples; no pooled estimate
Cortisol	*SMD*: 0.86 (95% *CI*: −0.04–1.76), $p = 0.06$	331 (4 studies)	⊕◯◯◯ very low	inconsistent results; PSG-based studies reported no significant differences
CgA	mixed findings: 2 studies reported higher salivary CgA levels, whereas one reported lower levels	176 (3 studies)	⊕◯◯◯ very low	small sample sizes, inconsistent findings, no pooled estimate
α-amylase	mixed findings: 1 study involving older adults reported a negative correlation with SB, whereas 1 study involving adults found no significant difference	264 (2 studies)	⊕◯◯◯ very low	conflicting findings, small sample sizes, no pooled estimate

CI – confidence interval; *OR* – odds ratio; *SMD* – standardized mean difference.

Discussion

This systematic review synthesized current evidence on the association between SB and salivary secretion, focusing on both quantitative (salivary flow rate) and qualitative (salivary biomarkers) aspects. The findings suggest a possible age-dependent pattern: SB in children and adolescents was more frequently associated with increased salivary flow, particularly sleep drooling, whereas in adults, SB was associated primarily with subjective sensations of dry mouth, possible reductions in salivary flow, and alterations in stress-related biomarkers.

Given that most of the included studies were observational, predominantly cross-sectional, and relied primarily on self- or parent-reported SB and subjective salivary outcomes, the reported associations should not be interpreted as evidence of causation. Therefore, the findings support only correlational inferences, and any causal relationships should be confirmed in longitudinal or experimental studies.

Sleep bruxism and salivary flow rate

The pooled data from this review demonstrated that children and adolescents with SB had significantly higher odds of drooling during sleep than non-bruxers (77.4% vs. 37.5%, *OR* = 2.88), although heterogeneity across studies was considerable and the certainty of evidence was very low. This finding is consistent with previous studies that have associated SB with immature orofacial motor regulation and frequent sleep arousals.[35,57] In addition, SB in children is frequently concomitant with disordered breathing conditions such as OSA, snoring, mouth breathing, and adenotonsillar hypertrophy, which may independently contribute to drooling by disrupting normal swallowing patterns and promoting saliva pooling.[19,25,26] In pediatric patients undergoing orthodontic or removable appliance therapy, excessive salivation may also lead to practical complications, including appliance instability, mucosal irritation and reduced treatment compliance.[58]

Nevertheless, these findings must be interpreted with caution. Most of the included studies relied on self-reported SB and drooling, limiting diagnostic validity and increasing the risk of recall and reporting bias. According to the latest international consensus (2025), the former grading system for SB diagnosis has been removed; instead, self-report, clinical examination and instrumental methods are each recognized as having specific strengths and limitations, with no single approach considered definitive.[1] Without objective confirmation using PSG or other instrumental measures, causal relationships cannot be established. The observed association may instead reflect shared developmental or neurophysiological processes during childhood and adolescence. Clinically, the coexistence of SB and hypersalivation may represent a potential marker of sleep-disordered breathing and may warrant further assessment, particularly in pediatric dentistry and orthodontic practice.

In adults, the association between SB and salivary flow remains uncertain. One study observed a slightly reduced, although not statistically significant, stimulated salivary flow rate in patients with SB,[55] whereas another study, using the Schirmer test to assess unstimulated morning salivary secretion, found no objective difference but reported a significant association between SB and the subjective sensation of dry mouth.[35] These discrepancies highlight the importance of distinguishing between objectively measured hyposalivation (an absolute reduction in salivary flow) and xerostomia (the subjective perception of oral dryness despite normal salivary flow). The latter may be particularly influenced by psychological stress and comorbid conditions such as OSA and snoring, both of which are commonly associated with SB.[22,23,59]

Despite these inconsistencies, one proposed explanation is that reduced salivation, whether objectively measured or subjectively perceived, might act as a physiological trigger for SB, with rhythmic masticatory muscle activity serving as a compensatory mechanism to stimulate saliva production.[15,55] However, this hypothesis remains speculative and has not been confirmed in high-quality studies. Additionally, because adults with OSA and snoring frequently report dry mouth,[24] the noted association between SB and OSA[60,61] may partly reflect shared underlying mechanisms related to salivary dysfunction. Correlations between SB, masticatory muscle pain and sleep-disordered breathing further support this integrated perspective.[11,59] These interrelated factors suggest that dental treatment planning, particularly for prosthodontic and implant-supported rehabilitation, should adopt an integrated approach that considers SB, salivary alterations and coexisting sleep-related conditions.

Sleep bruxism and salivary components

Several studies included in this review explored the relationship between SB and stress-related salivary biomarkers, including cortisol, CgA and α-amylase. Overall, the findings were inconsistent, with results varying across age groups, biomarker types and diagnostic methods. High heterogeneity, small sample sizes and the reliance on self-reported SB in most studies further reduced the certainty of the available evidence.

In children, the evidence regarding salivary cortisol is inconsistent. One earlier study involving 100 children (mean age: 7.2 years) reported significantly lower salivary cortisol levels in those with SB,[47] whereas a more recent study[46] involving a larger pediatric cohort (551 children aged 7–8 years) found elevated cortisol levels in individuals with SB. Both studies used comparable SB assessment methods (questionnaires combined with clinical examination) and cortisol assays (enzyme-linked immunosorbent assay (ELISA) or electrochemiluminescence), indicating that methodological differences are unlikely to account for the discrepant findings. Instead, they may reflect variations in participants' stress exposure, the timing of saliva collection or developmental differences in stress-regulation systems.

In adults, the meta-analysis of 4 studies showed higher salivary cortisol levels among participants with SB (*SMD*: 0.86, 95% *CI*: 0.44–1.29), although the association did not reach statistical significance. This finding is consistent with previous studies linking SB with salivary alterations, chronic stress, hypothalamic–pituitary–adrenal axis dysregulation, and bruxism-related symptoms.[20,28,30,34,46,62] Chronic stress may contribute to autonomic overactivation, salivary gland fatigue and reduced resting salivary flow,[22,23,57] which has been hypothesized to trigger SB as a compensatory mechanism aimed at restoring oral moisture through rhythmic masticatory muscle activity.

Findings regarding CgA and α-amylase were more variable. Two studies found a positive association between CgA and SB,[53,54] whereas one reported a negative association.[29] Regarding α-amylase, 1 study involving adults aged 25–55 years found no significant differences between bruxers and non-bruxers,[28] whereas another study involving older adults (>65 years) reported a negative correlation between SB and α-amylase levels.[31] These discrepancies may reflect the distinct physiological roles of these biomarkers, with cortisol representing chronic hypothalamic–pituitary–adrenal axis activation, and CgA and α-amylase reflecting more acute sympathetic nervous system activity. They may also be influenced by age-related differences in stress responsiveness and salivary gland function.[27,63–65]

However, it is important to note that 2 studies[54,56] using PSG for SB diagnosis reported no significant differences in salivary cortisol levels between patients with SB and controls. This discrepancy suggests that the reported association between SB and stress-related biomarkers may be influenced by the reliance on subjective stress measures and non-instrumental SB assessment methods. Consequently, associations observed in studies based primarily on self-reported SB should be interpreted with caution, as they may reflect shared psychological backgrounds rather than a direct physiological relationship.

Overall, although some evidence suggests an association between SB and stress-related salivary biomarkers, these findings should be interpreted with caution due to inconsistent results, possible age-related differences and substantial methodological limitations, particularly the reliance on non-instrumental methods for SB assessment.

Limitations

A major limitation of the included studies is the limited validity of the SB assessment methods. Only a minority of studies utilized instrumental diagnostic methods (PSG or EMG), while the majority relied on self-reported symptoms, parental reports or clinical signs such as tooth wear. However, self-report-based SB assessment is known to correlate only weakly with PSG-confirmed SB,[46] and tooth wear is not a specific indicator of SB[66,67] because it can result from multiple factors, including dietary habits, gastroesophageal reflux and parafunctional behaviors.

Because fewer than 10 studies were included in each meta-analysis, publication bias could not be formally assessed. Therefore, the presence of unpublished or selectively reported studies cannot be ruled out. Furthermore, this review was limited to English-language publications, introducing the risk of language bias and potentially excluding relevant studies published in other languages. In addition, blinding of outcome assessors was unclear in all included studies. This limitation is particularly important for subjective salivary outcomes (e.g., self-reported

xerostomia or drooling), where knowledge of SB status may have influenced outcome reporting and contributed to detection bias.

Notably, 6 of the pediatric studies originated from Brazil, suggesting that cultural, diagnostic or healthcare-system factors may have influenced both SB ascertainment and the reporting of salivary outcomes. This concentration of evidence may have inflated the pooled prevalence estimate of drooling in children with SB and limits the generalizability of findings to other populations.

This review also acknowledges that comorbidities such as OSA, gastroesophageal reflux disease and anxiety disorders may play an integral role in the observed associations between SB and salivary function.[10,11,59,64,68,69] Similarly, lifestyle factors such as smoking have been shown to alter salivary homeostasis and may therefore confound the relationship between SB and salivary parameters.[6,70] These conditions share overlapping neurophysiological and behavioral mechanisms with SB and may independently or synergistically influence salivary function. However, most included studies did not systematically assess or adjust for these potential confounders. Consequently, the reported associations may reflect the combined effects of coexisting conditions rather than a direct relationship between SB and salivary alterations.

Clinical implications

The systematic review highlights several clinical implications for the management of SB across different age groups. However, practical recommendations remain constrained by the heterogeneity of the available evidence and its overall very low certainty. Accordingly, clinical decision-making should remain individualized and conservative. Moreover, salivary biomarkers (cortisol, CgA, α-amylase) currently lack validated diagnostic utility and should not be used as standalone diagnostic markers for SB. When assessed, they should be regarded only as adjunctive, hypothesis-generating indicators. Emerging management frameworks for SB should therefore be interpreted in this context.[6,71–73] The main clinical implications are as follows:

- In pediatric populations, the observed association between SB and hypersalivation may complicate appliance therapy, potentially leading to reduced stability, mucosal irritation, or patient discomfort. Clinicians should therefore consider appropriate appliance modifications and reinforce oral hygiene measures to minimize saliva-related complications;
- In adults, SB may be associated with reduced salivary flow and/or subjective symptoms of dry mouth. The presence of subjective xerostomia, even in the absence of objectively measured salivary hypofunction, may reflect underlying stress-related autonomic dysregulation and should prompt evaluation for comorbid conditions such as OSA. These considerations are particularly relevant in prosthodontics and implant dentistry, where SB-related salivary alterations may affect prosthesis longevity and oral tissue health.

Future research directions

Future research should further elucidate the mechanisms underlying the relationship between SB and salivary function. In particular:

- The findings of this review suggest that the association between SB and salivary alterations differs between pediatric and adult populations, indicating the presence of age-specific mechanisms. Longitudinal studies with standardized assessment protocols are needed to confirm these findings and clarify the causal pathways linking stress, SB and salivary alterations;
- Future studies should adopt the most recent international consensus definitions of SB (2025)[1] and, whenever feasible, incorporate instrumental confirmation of SB episodes (e.g., PSG or EMG) together with objective and time-controlled saliva sampling protocols;
- The influence of comorbidities such as OSA, gastroesophageal reflux disease and psychological stress should be systematically controlled for or examined using stratified analyses in future studies;
- Interventional studies targeting salivary function or stress reduction may help determine whether modifying these factors can reduce SB severity or mitigate its oral health consequences;
- Future research should also investigate SB in populations with salivary disorders, such as xerostomia, Sjögren's syndrome and diabetes, as improved recognition and management of SB may contribute to better oral health outcomes and quality of life.

Conclusions

This systematic review suggests a potentially complex association between SB and salivary alterations. In children, SB appears to be more consistently associated with hypersalivation, whereas in adults, the findings are less consistent, with some evidence supporting an association with subjective dry mouth and elevated salivary cortisol levels, suggesting a possible stress-related mechanism. However, inconsistencies across studies, potentially due to differences in age groups, biomarker selection and the predominant reliance on self-reported SB assessment methods preclude firm conclusions. Future studies using objective SB diagnostic methods and standardized saliva collection protocols are needed to clarify these associations and to better inform clinical practice.

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

During the preparation of this work, the authors used ChatGPT (OpenAI, San Francisco, USA) to improve the wording and check the grammar. Subsequently, the authors reviewed and edited the work as needed and take full responsibility for the content of the publication.

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Review

Are gastrointestinal, gynecological and urogenital chronic overlapping pain conditions (COPCs) predisposing or perpetuating factors for orofacial pain (OFP)? A systematic review

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Abstract

Orofacial pain (OFP) is a multidimensional clinical problem that includes conditions such as temporomandibular disorders (TMD). It is often associated with chronic overlapping pain conditions (COPCs), including gastrointestinal, gynecological and urogenital disorders. Despite previous reviews, uncertainty remains regarding temporality, the effect size and evidence certainty. This systematic review and meta-analysis, registered in PROSPERO, synthesized observational studies assessing the association between COPCs and OFP. Twenty-two studies met the inclusion criteria, of which 12 were eligible for quantitative synthesis. The risk of bias (RoB) was assessed with the Quality in Prognosis Studies (QUIPS) tool, and the certainty of evidence was evaluated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. Longitudinal studies demonstrated, with moderate certainty, that irritable bowel syndrome (IBS) increases the risk of both first-onset and chronic OFP, including TMD and burning mouth syndrome. Cross-sectional evidence revealed associations of moderate magnitude between gastroesophageal reflux disease (GERD) and TMD, and large effects between polycystic ovary syndrome (PCOS) and TMD, though with lower certainty due to methodological limitations. Other conditions, such as interstitial cystitis (IC), gastritis, inflammatory bowel disease (IBD), and endometriosis, were supported by limited and low-certainty evidence. Overall, findings underscore the importance of considering COPCs in the clinical evaluation of OFP and support multidisciplinary approaches to management. Further high-quality longitudinal research is needed to establish causal relationships and inform mechanism-based treatment strategies.

Keywords: temporomandibular joint disorders, chronic pain, orofacial pain, facial pain, chronic overlapping pain conditions

Highlights

- Orofacial pain (OFP) is consistently associated with chronic overlapping pain conditions (COPCs), especially irritable bowel syndrome (IBS), gastroesophageal reflux disease (GERD) and polycystic ovary syndrome (PCOS).
- Evidence of moderate to low certainty suggests that IBS may increase the risk of developing both first-onset and chronic temporomandibular disorders (TMD).
- Early screening for COPCs is crucial in patients with OFP, both for clinical management and research purposes.
- Multidisciplinary management strategies that integrate dentistry, gastroenterology, gynecology and behavioral health are essential for improving outcomes in patients with OFP.

Introduction

Orofacial pain (OFP) refers to discomfort in the hard and soft tissues of the head, face and neck.[1] It may arise from dental, neurogenic, musculoskeletal, or psychophysiological origins, and is associated with cancer, infections, immune disorders, trauma, and stress.[1,2] Temporomandibular disorders (TMD) represent the most common chronic non-dental OFP condition. Recent data suggest that the global prevalence of TMD is currently estimated to be around 34%.[3] However, studies indicate that the overall prevalence of OFP in the general population ranges from 17% to 26%, with chronic forms affecting approx. 7–11% of adults.[4,5] Evidence shows women experience OFP nearly twice as often as men, with an odds ratio (*OR*) of 2.6 (95% confidence interval (*CI*): 2.5–2.7).[4] Estrogen levels influence pain modulation in the temporomandibular joint (TMJ) and the orofacial region. This hormonal effect may explain the higher prevalence of OFP in women; however, current evidence is insufficient to confirm a direct causal role of estrogen in TMD.[6,7]

Orofacial pain encompasses a wide range of clinical presentations and is characterized by its complexity, often reflecting the coexistence of multiple comorbidities.[8] The most common related conditions are primary myofascial pain syndrome and primary headaches. These are followed by irritable bowel syndrome (IBS) and chronic low back pain.[9] In this context, the presence of comorbidities highlights the multifactorial nature of OFP and the need to explore its relationship with other chronic pain conditions. Notably, IBS also shows a significant overlap with other urogenital pain disorders, such as interstitial cystitis (IC), painful bladder syndrome, chronic prostatitis (CP), and chronic pelvic pain (CPP). These conditions are commonly categorized as chronic overlapping pain conditions (COPCs), characterized by central sensitization and functional pain syndromes,[10] reflecting a complex neurobiological basis where altered nociceptive processing in the central nervous system plays a key role in symptom persistence.[11]

These findings emphasize the importance of distinguishing primary from secondary pain when examining OFP and identifying systemic conditions that may predispose to or perpetuate symptoms.[11] Recent evidence indicates that COPCs adversely affect the prognosis of chronic pain across various chronic diseases,[12] including OFP. Furthermore, a higher number of COPCs correlates with the prolonged duration and increased intensity of TMD.[13] Building on this evidence, understanding how these COPCs interact with OFP may provide insight into the shared mechanisms underlying pain chronification. Conditions like IBS often co-occur with TMD and share common risk factors,[14] including psychological elements, such as anxiety.[15] Similarly, chronic secondary pain[11] stemming from identifiable systemic pathologies, such as autoimmune or inflammatory diseases, has been linked to orofacial manifestations.[11] For example, inflammatory bowel disease (IBD) has been associated with bruxism and TMD.[16]

In addition, sleep disturbances and parafunctional behaviors, such as sleep bruxism, frequently overlap with OFP and other COPCs, further complicating diagnosis and prognosis. Polysomnographic evidence indicates that individuals with OFP and headache complaints often exhibit reduced sleep quality and altered sleep architecture, which may exacerbate pain perception and contribute to central sensitization processes.[17] Moreover, recent findings show that different general health conditions may aggravate sleep bruxism–related symptoms and pain perception in patients with TMD.[18] These results reinforce the notion that impaired sleep regulation and systemic factors act synergistically in sustaining chronic pain, supporting the inclusion of sleep and behavioral assessments in the comprehensive evaluation of OFP.

Despite systematic reviews exploring the relationship between TMD and COPCs, important methodological and conceptual gaps persist. Building upon the previous context, it becomes evident that current summaries do not yet provide a clear picture of temporality or risk. Based on the preliminary search on this topic, and the reviews identified via Epistemonikos (the biggest database for systematic reviews) and PubMed, current evidence syntheses remain limited. Most reviews[9,19–22] are purely descriptive, providing only qualitative summaries. Longitudinal studies are underrepresented, and key aspects investigating the association between OFP (especially TMD) and COPCs, such as temporality, the effect size and risk estimation, are often missing.

Kleykamp et al.[9] and Moisset et al.[20] reported comorbidity prevalence using cross-sectional designs without addressing temporality or prognostic relevance, while Warzocha et al. focused on statistical significance without reporting effect sizes from the associations or study design differences among the included studies.[22] In addition, although Da-Cas et al. incorporated the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to evaluate the certainty of the overall evidence, their review remained qualitative without meta-analyses or stratified analyses.[19] Furthermore, other reviews also relied heavily on secondary analyses from the same cohort (OPPERA), potentially inflating certain findings. Moreover, diagnostic criteria for TMD and other OFP conditions have been inconsistently applied in the existing reviews.[20,23] This review addresses these gaps by including both cross-sectional and longitudinal studies that meet validated OFP diagnostic criteria, incorporating risk assessment, evaluating the certainty of evidence using GRADE, and performing quantitative meta-analyses where possible. In doing so, it provides the first quantitative and certainty-based synthesis of COPC–OFP associations, establishing both risk and temporality.

We hypothesized that pain-related gastrointestinal, gynecological and urogenital conditions may act as risk or prognostic factors for OFP through mechanisms such as central sensitization, hyperalgesia and low-grade inflammation. Therefore, our main objectives for this review are as follows:

– to compile and synthesize the evidence on the relationship between COPCs and OFP;
– to determine whether these COPCs act as risk factors for the onset of OFP or as prognostic factors for the chronification of OFP;
– to estimate, when possible, the magnitude and direction of these associations; and
– to identify gaps in the literature, and inform future research and clinical practice.

Methods

Protocol and registration

The protocol of the study was registered with the International Prospective Register of Systematic Reviews – PROSPERO (registration No. CRD42024537553). The review was conducted and reported following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[24]

Eligibility criteria

This review examines the association between OFP and pain-related gastrointestinal, gynecological and urogenital disorders (COPCs). The inclusion and exclusion criteria were defined using the adapted Population, Predictor/Prognostic Factors, and Outcomes (PFO) framework.[25]

Population

The inclusion criteria comprised studies targeting adults (aged ≥18 years) diagnosed with acute, subacute or chronic OFP, TMD or intraoral pain by a clinician, based on symptoms, using standardized diagnostic tools, such as the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD),[26] the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD)[27] or other recognized instruments (the International Classification of Orofacial Pain, 1st edition (ICOP-1),[28] the International Classification of Headache Disorders (ICHD-1,[29] ICHD-2[30] or ICHD-3[31])). All OFP subtypes were eligible. Diagnoses followed ICOP[28] and included: OFP associated with TMD (myofascial pain, arthralgia, or headache attributed to TMD); orofacial neuropathic pain (classical trigeminal neuralgia); trigeminal neuropathy (postherpetic neuropathy, numb chin syndrome, burning mouth syndrome (BMS)); central and idiopathic neuropathic pain (persistent idiopathic facial pain, occlusal dysesthesia); and intraoral pain (pulpal or periodontal pain). Full definitions are detailed in the supplementary material (S1A) (all supplementary materials are available from the corresponding author upon reasonable request).

Studies including both clinical (outpatient or hospital-based) and non-clinical (community or population-based) samples were eligible, provided that diagnostic criteria were clearly defined and validated. Studies were excluded if they involved pregnant participants, due to the typical exclusion of pregnancy in TMD and OFP research because of hormonal and physiological influences on pain outcomes.

For analytical consistency, diagnoses were primarily grouped into TMD pain-related conditions, including studies using RDC/TMD, DC/TMD other validated diagnostic instruments, or structured clinical examinations consistent with these criteria. When this grouping was not possible due to differences in diagnostic scope or reporting, studies were classified into the broader OFP category. The OFP group encompassed diagnoses based on ICOP, ICHD or DC/TMD (when integrated within the ICOP definitions) or structured clinical examinations. This approach ensured methodological rigor while preserving diagnostic comparability across heterogeneous study designs.

The exclusion criteria comprised studies involving animal models, terminally ill patients, cancer-related pain, or pediatric populations. Studies lacking validated diagnostic criteria or a recognized structured clinical framework for diagnosing OFP were also excluded.

Predictors/prognostic factors of interest

The included studies should have investigated the association between OFP conditions and the COPCs affecting

the gastrointestinal, gynecological and urogenital systems. These were considered regardless of whether they represented chronic primary pain (without identifiable structural pathology) or chronic secondary pain (linked to clear organic findings).

The diagnostic confirmation of these conditions relied on clinical evaluation, including surgical, radiologic, endoscopic, or histopathological findings,[16] and disease markers in biopsy samples.[32] Validated self-report tools were accepted, including the Rome III[33] and IV[34] criteria, the bowel endometriosis syndrome (BENS) score,[35] the genitourinary pain index (F-GUPI/M-GUPI), the lower urinary tract dysfunction research network symptom index-29 (LURN SI-29),[36] and other structured clinical assessment tools. Patients were included whether OFP preceded or followed the onset of COPCs, recognizing their bidirectional interaction. While key conditions are outlined below, the review included any pain-related gastrointestinal, gynecological or urological disorder linked to OFP.

In the gastrointestinal domain, IBS and gastroesophageal reflux disease (GERD) represent the most prevalent disorders, whereas IBD, although less frequent, carries a disproportionate clinical and healthcare burden. Irritable bowel syndrome is a chronic primary disorder involving abdominal pain and altered bowel habits, while IBD includes chronic relapsing inflammatory conditions like Crohn's disease and ulcerative colitis.[37,38] Gastroesophageal reflux disease occurs when stomach contents reflux into the esophagus, causing symptoms or complications.[39] Common symptoms are heartburn and regurgitation. Gastroesophageal reflux disease may cause extraesophageal manifestations like cough, laryngitis and dental erosion. Complications include esophagitis, strictures, Barrett's esophagus, and adenocarcinoma.[40]

Primary dysmenorrhea, defined as menstrual pain in the absence of pelvic pathology, is the most prevalent gynecological complaint, particularly among women of reproductive age.[41] Abnormal uterine bleeding and chronic estrogen-dependent conditions with ectopic endometrial-like tissue and pain[42] are commonly identified. Bleeding can relate to structural disorders like polyps, adenomyosis, leiomyoma, malignancy, and hyperplasia, or anomalies such as coagulopathy, ovulatory dysfunction or endometrial pathology.[42] Endometriosis is an inflammatory estrogen-dependent disease associated with pelvic pain and/or infertility, characterized by uterine and extrauterine tissue lesions.[32]

Urological pain syndromes were considered within the broader category of CPP, particularly when pain was perceived in the bladder, urethra, prostate, penis, or scrotum, and no active infection or structural cause was present.[43] These chronic primary urological syndromes often present with lower urinary tract symptoms, sexual dysfunction and psychosocial impact. Examples include primary bladder pain syndrome, prostate pain syndrome, and urethral, penile and scrotal pain syndromes.

Chronic overlapping pain conditions were classified according to the framework established by the International Association for the Study of Pain (IASP) and the International Classification of Diseases 11th revision (ICD-11),[44] as well as the guidelines from the European Association of Urology (EAU).[43] These guidelines are conceptually based on the IASP classification of chronic pain, which differentiates between chronic primary pain syndromes (such as IBS and primary dysmenorrhea) and chronic secondary pain syndromes (such as IBD and endometriosis). Full definitions are provided in the supplementary material (S1B).

Outcomes of interest

The primary outcomes were OFP development/progression and its association with the COPCs described above. We evaluated whether these conditions were associated with OFP, and quantified the strength of these associations. The progression of OFP was operationalized according to the clinical indicators reported in the included studies, including increases in pain intensity (e.g., the numeric rating scale (NRS) or visual analog scale (VAS) scores), symptom frequency or duration, and the clinical transition between acute, subacute and chronic conditions. The associations between TMD and IBS were further examined in relation to IBS severity scores, the number of gastrointestinal symptoms, and TMD-related pain duration or intensity.

Effect sizes for the associations were determined using univariate models (e.g., Pearson's or Spearman's correlations, *OR*, the relative risk (*RR*)) or multivariable models (e.g., logistic regression, Cox regression, Poisson regression), providing estimates such as *OR*, *RR* or the hazard ratio (*HR*). When only prevalence data were reported, yet crude *OR* could be calculated, we did so, distinguishing these from the adjusted models for exploratory inferences.

Study designs

Due to the nature of our question (i.e., looking for an association), we targeted mainly observational studies. Retrospective and prospective cohort studies, case–control studies, cross-sectional studies, and case series were included. Controlled observational studies with healthy participants and association statistics were included. Non-controlled cohort studies with data to estimate associations were also included. We excluded randomized controlled trials (RCTs), controlled trials, case reports, reviews, systematic reviews, meta-analyses, commentaries, letters, conference papers, book chapters, protocol registrations, and abstracts without full texts, as these designs would not appropriately address our question. Relevant references in the excluded reviews were screened for inclusion. We only included original data, and excluded secondary analyses from the same study data or database as primary studies, e.g., multiple publications using the same data from the OPPERA cohort.

Review question

The review question was as follows: "Can any gastrointestinal, gynecological and urogenital COPCs be considered a risk/prognostic factor for the development and/or progression of OFP?"

Search strategy

A health sciences librarian with 20 years of experience conducted a computerized literature search as part of a research project investigating the associations between COPCs and OFP, headache and neck pain. The search strategy was designed to comprehensively capture studies across all 3 pain domains.

The search was conducted initially in 2023, and then updated again on July 29, 2025, for this specific research question. The following databases were searched: Ovid MEDLINE (1946 to July 29, 2025); CINAHL (from inception up to July 29, 2025); Embase (1974 to July 29, 2025); Scopus (1927 to October 20, 2023); and the Web of Science (WoS) (1976 to July 29, 2025). The subject headings and keywords were adapted for each database. The search strategy focused on terms (Medical Subject Headings (MeSH) and free-text) related to OFP and COPCs, as well as their associations. No time or language restrictions were imposed.

In addition, PROSPERO was searched to identify ongoing or unpublished systematic reviews on the topic. ClinicalTrials.gov was excluded, as it primarily indexes interventional trials, which were not relevant to the present research question. OpenGrey was not included, since its database has not been updated since 2020.

For this review, only studies reporting on the relationship between OFP and any of the COPCs listed above were included. During full-text screening, the reference lists of eligible articles were reviewed to identify additional studies. Backward and forward citation tracking was performed through WoS on July 17, 2025, to identify additional or unpublished studies. Duplicate records from the overlapping databases were automatically detected and removed using the Covidence tool (https://www.covidence.org) before the screening process. The full search strategies conducted in all databases initially, and when updating the search, are available in the supplementary material (S2).

Study selection

The search results were imported into EndNote and transferred to Covidence for screening. We used Covidence to track the review process, depicted in the last version (2020) of the PRISMA flowchart.[24] Three reviewers (CJM, JBS, MSB) independently screened the titles and abstracts according to the predefined eligibility criteria. Full texts were reviewed for the studies meeting the inclusion criteria or where eligibility was unclear from the abstracts. For studies with multiple publications, the most recent or complete versions were included. The reviewers were blinded to each other's decisions to reduce bias. Discrepancies were resolved by consensus, with a third reviewer (senior author SAO) making the final decisions if needed.

Data extraction

Data extraction was conducted using a customized form in the Covidence software. A standardized data extraction form was applied and refined through discussions to ensure consistency. Dropdown menus helped to standardize responses. Reviewers received training. Four reviewers (CJM, JIO, HvdM, GAB) independently extracted data, and one reviewer (CJM) compiled the consensus information into Excel tables. Discrepancies were resolved by consensus; if no agreement was reached, the lead author decided (SAO).

The risk of bias (RoB) was assessed by 2 independent reviewers (CJM, HvdM) using the Quality in Prognosis Studies (QUIPS) tool.[45] This tool evaluates 6 domains: study participation; study attrition; prognostic factor measurement; outcome assessment; study confounding; and statistical analysis/reporting. In each domain, the RoB ratings were performed based on the following decision rules: (1) high RoB – when a domain showed serious methodological concerns likely to bias associations (e.g., poor participation, high attrition, inadequate measurements, confounding not controlled, or flawed analysis); (2) moderate RoB – when evidence was inconclusive to classify a domain as high or low RoB; and (3) low RoB – when most criteria were methodologically sound and bias was unlikely.[45] The overall RoB judgment followed Hayden et al.[46]: (1) high RoB – at least one domain rated as high; (2) moderate RoB – maximum 2 domains rated as moderate, with others low; and (3) low RoB – all domains rated as low.

Strategy for statistical analysis and data synthesis

Random-effects meta-analyses were conducted using the metafor[47] package for the R software, applying an inverse variance model with the restricted maximum likelihood (REML) estimation. Analyses were performed for each comorbidity pair, using log odds ratios (log (*OR*)) and standard errors (*SE*). Summary estimates, 95% *CI*, and heterogeneity statistics (I^2 and τ^2) were reported. Adjusted and unadjusted data were analyzed separately to account for confounding factors.

To ensure analytical consistency, studies were grouped by methodological design into longitudinal and cross-sectional categories, reflecting the temporal design rather than RoB. Differences in study quality, diagnostic criteria and analytical adjustments were evaluated via the leave-one-out (LOO) sensitivity analyses to assess the influence on the pooled estimates and heterogeneity.

No conversions between effect measures (e.g., *OR*, *RR*, *HR*) were performed; each meta-analysis included studies reporting the same measure type. When studies did not report *OR*, but provided raw 2×2 data, crude *OR*s were calculated using the MedCalc *OR* calculator[48] for inclusion in relevant meta-analyses.

The LOO sensitivity analyses assessed how each study influenced the pooled effect and heterogeneity. For each LOO iteration, one study was removed and a new random-effects model was recalculated. Meta-analyses were considered unstable if removing a study changed the *CI* from excluding to including the null value (*OR* = 1) or reversed the effect direction. Following methodological guidance,[49,50] studies compromising stability due to high heterogeneity or low quality were excluded from the final model. Forest plots were generated using ggplot2[51] to display the individual and pooled estimates with effect sizes, GRADE ratings, and color-coded diagnostic groups. Analyses were performed in R, with outputs included in the manuscript and the supplementary materials.

Effect sizes for ratio measures (*OR* and *RR*) were interpreted using the guidelines by Chen et al.[52] The authors related *OR* to Cohen's *d* by comparing standardized probability differences. Their estimates showed that *OR* values of 1.68, 3.47, and 6.71 corresponded to small (*d* = 0.2), medium (*d* = 0.5), and large (*d* = 0.8) effect sizes when the baseline outcome rate was 1% in the non-exposed group.[52] In this review, *OR* or *RR* values ≤1.68 were considered negligible, 1.68–3.47 small, 3.47–6.71 moderate, and ≥6.71 large. For hazard ratios, we followed Vibha et al.[53]: *HR* ≤ 1 indicated no increased risk; 1.2–1.5 moderate risk; and >1.5 high risk. For unstandardized β coefficients from linear regression models, effect sizes were interpreted relative to the outcome scale and the established thresholds of clinical importance. For pain intensity measured with NRS (0–10), coefficients predicting changes of about 1.90 points were considered minimally clinically important,[54] whereas changes of ≥2 points were regarded as clinically relevant. This approach allowed a patient-centered interpretation of effect sizes. For pain duration in years, changes exceeding 1 year were substantial when the baseline duration was approx. 10 years. This approach allowed a patient-centered interpretation of effect sizes.

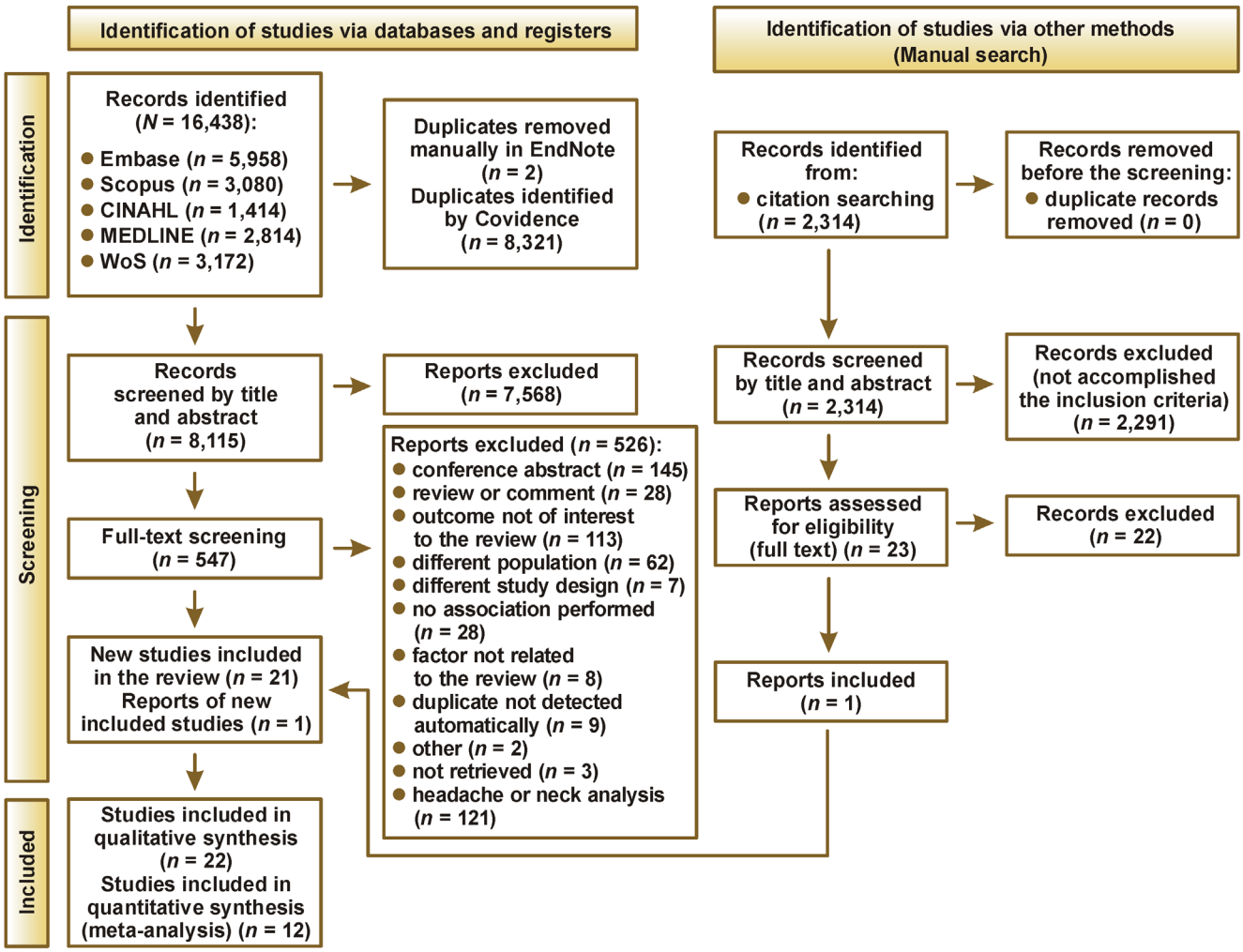


Fig. 1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flowchart of the study

Certainty of evidence

The quality of prognostic evidence was assessed using the GRADE approach. Following the criteria by Huguet et al.,[55] the certainty of evidence was rated as high (⊕⊕⊕⊕), moderate (⊕⊕⊕◯), low (⊕⊕◯◯), or very low (⊕◯◯◯). For each analysis, we evaluated the following: (1) the number of participants; (2) the number of cohorts; (3) study limitations; (4) result inconsistency; (5) indirectness (limited generalizability); (6) imprecision (insufficient or wide *CIs*); (7) publication bias risk; (8) effect magnitude; and (9) the presence of a dose–response gradient.

Outcomes of interest were grouped by study design (longitudinal vs. cross-sectional) and based on the analysis performed. The GRADE assessment was conducted for each of these groups using the approach by Huguet et al.[55] Longitudinal studies provided prognostic evidence, while cross-sectional studies contributed association evidence. For each TMD–OFP/COPC pair, certainty ratings were synthesized considering all domains of GRADE – the study design, RoB, inconsistency, indirectness, and imprecision.

Results

Study selection

The PRISMA flowchart in Fig. 1 shows the selection process. After compiling the data from the search of databases, 16,438 preliminary studies were found. After removing duplicates, 8,115 studies remained, and 547 were read fully. After reviewing all manuscripts, 121 were excluded, but still considered for other analysis related to the headache and neck pain project. Of the remaining studies, 22 met the inclusion criteria,[13,16,56–75] and 12 were included in the meta-analysis. Details regarding the reasons for exclusion are provided in the supplementary material (S3).

Study characteristics and variables

We divided the studies into 2 groups. The 1st group includes studies that enable the investigation of the temporality of conditions and factors (temporality-oriented studies).[66,68,70,73] The 2nd group comprises studies adopting a cross-sectional approach or not accounting for temporal dimensions (cross-sectional associative studies).[13,16,56–65,67,69,71,72,74,75] Below, we report variables that were classified under each group. Detailed results related to these variables are presented in Table 1.

The general characteristics of the studies are summarized in Table 2. Most studies presented a high RoB, 6 studies were rated as having a moderate RoB, and only 2 could be classified as having a low RoB. For a summary of the RoB of these studies, refer to Fig. 2.

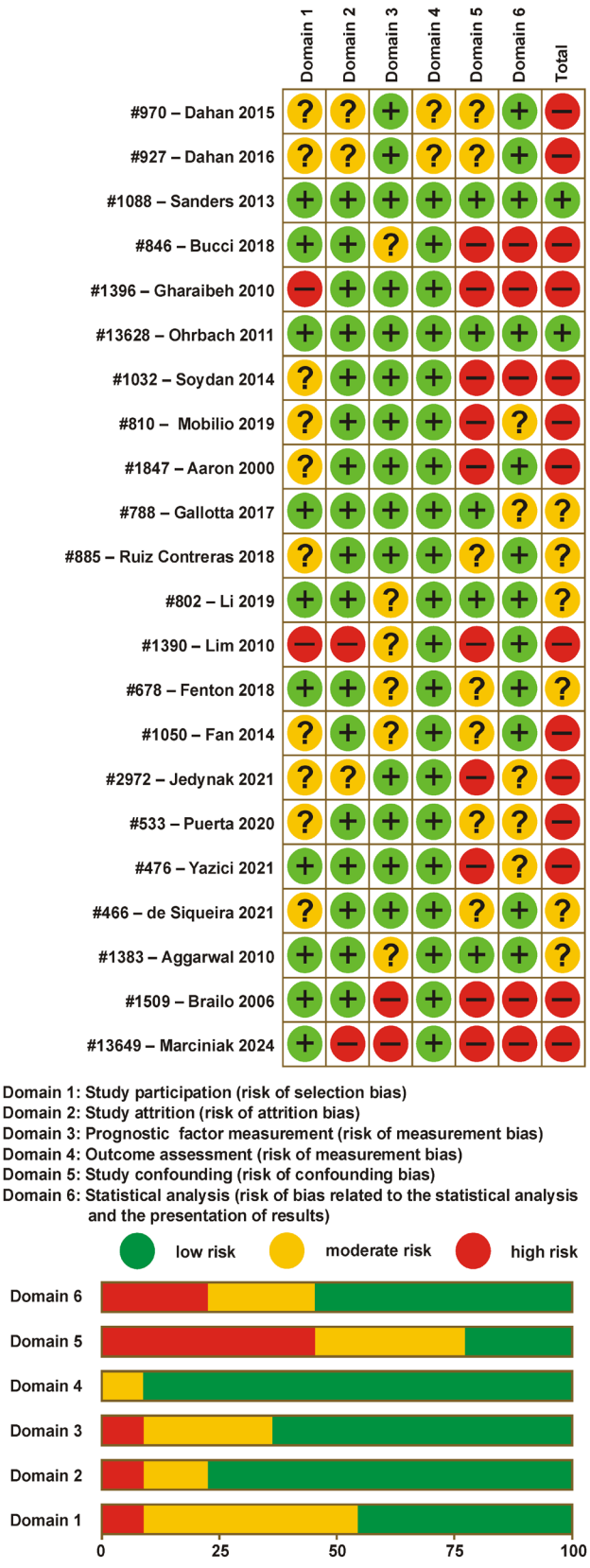


Fig. 2. Risk of bias (RoB) assessment using the Quality in Prognosis Studies (QUIPS) tool

The left panel displays individual study ratings across each evaluated domain, classified as high (red, –), moderate (yellow, ?) or low (green, +) RoB. The right panel summarizes the proportion of studies in each category per domain. Colors and symbols follow a traffic-light format to facilitate rapid visual interpretation.

Table 1. Summary of the results

	Factor	Studies (Study population/ Method/ RoB)	Condition definition / Exposure level
Predictor / prognosis variables (First-onset TMD or Chronic TMD vs. Controls without pain). Primary disorders	1.Likelihood of first-onset TMD among subjects with IBS	Sanders 201370 (N total: 2706) (N total: 2706) Prospective cohort (IBS before First-onset TMD). Community or population-based In total sample (N total:2706), IBS was identified first (74 subjects with IBS; 194 without IBS) and then those were followed and first onset TMD identified (N:268). Low risk of bias	IBS defined by self-reported questionnaire including Rome III criteria. Prevalence and likelihood of general IBS among first-onset TMD subjects. 74 subjects with IBS; 194 without IBS
	2. Likelihood of first-onset TMD among subjects with dysmenorrhea	Lim 201066 (N total: 266) Prospective cohort (Menstrual pain before chronic TMD). Community or population-based In total sample (N:266), dysmenorrhea was identified first and then those were followed and first onset TMD identified (16 subjects developed TMD). High risk of bias	All participants complete the Symptom Report Questionnaire (SRQ), assessing menstrual pain symptoms over a 3-year observation period prior to TMD onset. SRQ categories: "Not at all," "A little bit," "Moderate amount," "Quite a bit," and "An extreme amount."
	3. Likelihood of chronic OFP among subjects with IBS	R. Ohrbach 201168 (N total: 1802) Nested case-control study within a prospective cohort (IBS before chronic TMD). Community or population-based In total sample (N total:1802), A total of 185 developed chronic TMD and 1601 not developed chronic TMD. IBS was identified first (16 subjects with IBS; 168 without IBS). Prevalence of IBS was evaluated in subjects with chronic TMD compared to healthy controls. Low risk of bias	IBS diagnosed with validated self-reported questionnaire (Rome III). Subjects with chronic TMD: 16 subjects with IBS, 168 subjects without IBS. Controls: 43 subjects with IBS, 1558 subjects without IBS
		Aggarwal 201073 (N total: 1206) Prospective cohort (IBS before chronic Orofacial pain). Community or population-based In total sample (N:1206), IBS was identified first (N with IBS:106, without IBS: 1100). 10 Subjects with IBS developed chronic OFP and 46 without IBS developed chronic OFP. Moderate risk of bias	IBS diagnosed with validated self-reported questionnaire (Rome II). Subjects with Chronic Orofacial Pain: 10 YES, 46 NO Controls: 96 YES, 1054 NO
Association variables (subjects with specific diagnosis vs. Controls without pain). Exposure to primary disorders	4.Bidirectional association between TMD and IBS	Mobilio 202067 (N total: 82) Cross-sectional (no temporal analysis) Clinical sample In total sample with TMD (N: 82), IBS was identified at the present time (N with IBS: 26, without IBS: 56). Prevalence of IBS was evaluated in subjects with TMD compared to healthy controls. High risk of bias	IBS diagnosed via self-reported questionnaire (Rome III). IBS prevalence in TMD vs. healthy controls. Subjects with TMD: 22 YES, 25 NO. Controls: 4 YES, 31 NO.
		Gallotta 201762 (N total: 148) Cross-sectional (no temporal analysis) Clinical sample In total sample with IBS (N:148), TMD was identified at the present time (N with TMD:65, without TMD: 83). Prevalence of TMD was evaluated in subjects with IBS compared to healthy controls. Moderate risk of bias	TMD diagnosis via clinical exam (RDC/TMD). Prevalence among IBS vs. healthy controls. Subjects IBS: 50 YES, 41 NO. Controls: 15 YES, 42 NO.
	5. Association between IBS and Chronic TMD	Fenton 201861 (N total: 4.238.788 total) Retrospective cohort (no temporal analysis) Nationwide clinical registry In total sample with chronic TMD recruited retrospectively from a dataset (N: 4.238.788 total) chronic IBS was identified to be also present (N with IBS:20168, without IBS: 4106820). Prevalence of IBS was evaluated in subjects with chronic TMD compared to healthy controls. Moderate risk of bias	IBS defined by ICD codes (≥2 outpatient or ≥1 inpatient visit) Subjects with chronic TMD: 200 YES, 12426 NO Controls: 19968 YES, 4.094.394 NO
		Dahan 201658 (N total: 180) Cross-sectional (no temporal analysis) Clinical sample In total sample with TMD (N chronic: 180), IBS was identified at the present time (N with myofascial pain TMD: 51, N with non-myofascial pain TMD: 14). Prevalence of IBS was evaluated in subjects with chronic myofascial pain TMD compared to no miofascial pain TMD controls. High risk of bias	IBS diagnosed via validated self-reported questionnaire (Rome III) Subjects with TMD: 51 YES, 129 NO Subjects with m-TMD: 37 YES, 84 NO Subjects with n-TMD: 14 YES, 45 NO
		Aaron 200056 (N total: 47) Cross-sectional (no temporal analysis) Clinical sample In total sample (N: 47), IBS was identified at the present time (N with IBS: 20, N without IBS: 27). Prevalence of IBS was evaluated in subjects with chronic TMD compared to healthy controls. High risk of bias	IBS diagnosed by questionnaire + clinical assessment (Manning criteria) Subjects with chronic TMD: 16 YES, 9 NO Controls: 4 YES, 18 NO
	6. Association between IC and chronic TMD	Dahan 201658 (N total: 180) Cross-sectional (no temporal analysis) Clinical sample In total sample with chronic TMD (N: 180), IC was identified at the present time (N with myofascial TMD: 17, N with non-myofascial TMD: 5). Prevalence of IC was evaluated in subjects with chronic myofascial pain TMD compared to no miofascial pain TMD controls. High risk of bias	IC diagnosed via self-reported Pain, Urgency, and Frequency Symptom Scale IC prevalence: Subjects with All TMD: 22 YES, 158 NO Subjects with m-TMD: 17 YES, 104 NO Subjects with n-TMD: 5 YES, 54 NO
		Aaron 200056 (N total: 47) Cross-sectional (no temporal analysis) Clinical sample In total sample (N: 47), IC was identified at the present time (N with IC: 20, N without IC: 27). Prevalence of IBS was evaluated in subjects with chronic TMD compared to healthy controls. High risk of bias	Chronic IC diagnosed by 138-item self-reported questionnaire Subjects with chronic TMD: 4 YES, 21 NO Controls: 0 YES, 22 NO
		Fan 201460 (N total: 244) Cross-sectional (no temporal analysis) Clinical sample In total sample with (N:244), IC was identified at the present time (N with IC:15, without IC: 229). Prevalence of IC was evaluated in subjects with chronic TMD compared to healthy controls. High risk of bias	IC diagnosed using 2009 IC/HBS clinical guidelines Subjects with chronic TMD: 9 YES, 113 NO Controls: 6 YES, 116 NO
	7. Association between Gastritis and chronic Orofacial Pain	Puerta 202069 (N: 306) Clinical sample Cross-sectional (no temporal analysis) In total sample with (N:306), Gastritis was identified at the present time (N with Gastritis:58, without Gastritis: 148). High risk of bias	Gastritis diagnosed by physicians from hospital clinics using appropriate diagnostic protocols (clinical examination and other assessments as necessary). Subjects with chronic Orofacial pain: 38 YES, 136 NO Controls: 20 YES, 112 NO
		Contreras 201857 (N: 352) Cross-sectional (no temporal analysis) Clinical sample In total sample with (N:352), chronic Gastritis was identified at the present time (N with Gastritis:102, without Gastritis: 69). Prevalence of Gastritis was evaluated in subjects with chronic TMD compared to healthy controls Moderate risk of bias	Chronic gastritis/peptic ulcer (≥6 months), self-reported questionnaire Subjects with chronic TMD: 96 YES, 209 NO Controls: 6 YES, 41 NO Comp. 1: 13 YES, 28 NO Comp. 2: 81 YES, 181 NO
		De Siqueira 202159 (N total: 306) Retrospective Case-Control (no temporal analysis) Clinical sample In total sample with (N:306), chronic gastric complaints were identified at the present time (N: 98). Prevalence of gastritis was evaluated in subjects with chronic orofacial pain compared to healthy controls Moderate risk of bias	Systematized clinical evaluation of the Subjects. In addition, chronic Gastric complaints (≥6 months), and sensation of dry mucosa and xerostomia, assessed by the Xerostomia Inventory, validated to the Portuguese language. Subjects with chronic OFP: 65 YES 109 NO Controls: 33 YES 99 NO
		Brailo 200675 (N total: 156) Case-Control (no temporal analysis) Clinical sample In total the total sample some had true BMS (N:76), previous diagnose of Gastritis was identified at the present time (N with Gastritis: 61, without Gastritis: 95). Prevalence of Gastritis was evaluated in subjects with BMS compared to non BMS controls High risk of bias	Self-reported previous diagnose by a specialist of gastritis Subjects with Burning Mouth: 39 Yes 37 No Control 22 Yes 58 No

Subjects' diagnosis definition / Exposure level	Statistical analysis	Individual results 95% CI	Results in meta-analysis / Certainty
First-onset TMD diagnosed using RDC/TMD criteria. ≥5 days/month of TMD-related pain confirmed by examiner findings (arthralgia/myalgia).Both sexes included. N: 268 Control group: healthy participants who did not develop TMD. N: 2438	1.Adjusted multivariable analysis using random forest modelling (HR, CI). 2.Adjusted Cox proportional hazards regression model to assess increased incidence of TMD (RR, CI).	1.HR 1.62* [0.94–2.81] p < 0.001 Likelihood of developing first-onset TMD after developing IBS Moderate effect size 2. RR 2.27¥ [1.35–3.79] p < 0.001 Association between prior IBS and incidence of first-onset TMD Small effect size	Not included in meta analysis ⊕⊕⊕◯ Moderate
First-onset TMD diagnosed using RDC/TMD criteria. Female individuals. N: 16 Control group: healthy participants who did not develop TMD. N: 250	Repeated measures ANOVA between-group mean difference (Time × Group interaction) Only the p-value for the Time × Group interaction could be extracted, as the figure did not provide the exact effect size.	p 0.0036 Participants who developed TMD reported more menstrual pain (P 0.0036) than participants who did not develop TMD at both the baseline and the final visits.	Not included in meta analysis ⊕◯◯◯ Very low
TMD chronic (≥6 months), RDC/TMD criteria, ≥5 days/month of pain + examiner-confirmed arthralgia or myalgia. Both sexes included. N: 185 Healthy controls. N: 1601	Adjusted logistic regression (OR, CI)	OR 2.7§ [1.4–5.1] p <0.0001 Likelihood of developing chronic TMD after developing IBS. Small effect size	OR 2.32 [1.43–3.76] ⊕⊕◯◯ Low
Chronic Orofacial pain (for one day or longer in the past month and ≥3 months), subjects through validated self-reported questionnaire (Classification Questionnaire for Orofacial Pain). Orofacial pain was defined as pain in the face, mouth or jaws. This definition is likely to have included TMD, facial neuropathies, BMS, atypical odontalgia. Both sexes included. N: 56 Control are subjects that does not develop chronic orofacial pain. N: 1150	Adjusted multivariable analysis model (OR, CI).	OR 1.9§§ [0.9–3.9] p <0.001 Likelihood of developing chronic OFP after developing IBS. Small effect size	
TMD based on RDC/TMD criteria. Both sexes included. N: 47 Healthy subjects as controls. N: 35	Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 6.82 [2.08–22.38] p 0.0015 Association between subjects with TMD and IBS. Large effect size	OR 4.11 [2.22– 7.61] p <0.001 Moderate effect size ⊕⊕◯◯ Low
IBS diagnosed using Rome III + physical exam, blood tests, sigmoidoscopy, and other diagnostics. Both sexes included. N: 91 (IBS), Healthy controls N: 57	Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 3.41 [1.66–7.01] p 0.001 Association between subjects with IBS and TMD Moderate effect size	
Chronic TMD identified using ICD codes (≥2 visits in 18 months). Both sexes included. Comp. 1: TMD Female. N: 2844 Comp. 2: TMD Male. N: 9782 Healthy subjects as controls.	1. Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups for all subjects (all) 2. Adjusted multivariable analysis for female (CI OR) 3. Adjusted multivariable analysis for male (CI OR)	1. TMD all OR 3.30 [2.86–3.79] p < 0.0001 Association between subjects with chronic TMD and IBS Moderate effect size 2.TMD Female OR 1.67£ [1.36–2.04] Association between subjects with chronic TMD Female and IBS Small effect size 3.TMD Male OR 1.84£ [1.50–2.24] Association between subjects with chronic TMD Male and IBS Small effect size	OR 1.72 [1.42–2.06] p < 0.001 Small effect sice ⊕⊕◯◯ Low
Chronic TMD with mean duration 6.3 ± 0.6 years (RDC/TMD). Both sexes included. Comp. 1: m-TMD (myofascial pain only) Comp. 2: n-TMD (non-myofascial pain)	Adjusted logistic regression (CI OR)	m TMD OR 1.15‡ [0.52–2.55] Association between subjects with chronic TMD related to myofascial pain and IBS. Small effect size	
Chronic TMD (≥3 months), diagnosed using RDC/TMD criteria. Both sexes included. N: 25 Healthy subjects as controls. N: 22 controls	Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 8 [2.55–37.43] p 0.0027 Association between subjects with chronic TMD and IBS Large effect size Excluded from Meta-analysis: Not adjusted for confounders as the others and high risk of bias	
TMD (mean duration: 6.3 ± 0.6 years), diagnosed using RDC/TMD criteria. Both sexes included. Comp. 1: m-TMD (myofascial pain only) Comp. 2: n-TMD (non-myofascial pain)	Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	m TMD OR 1.76 [0.52–5.04] Small effect size m TMD OR 1.06‡ [0.31–3.59] Association between subjects with chronic TMD related to myofascial pain and IC Small effect size	OR 1.63 [0.75–3.55] p 0.12 Small effect size ⊕◯◯◯ Very low
TMD chronic (≥ 3 months), RDC/TMD criteria. Both sexes included. Both sexes included. N: 25 Healthy subjects as controls. N: 22	Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 9.4 [0.47–185.6] p 0.14 Association between subjects with chronic TMD and IBS large effect size Excluded from Meta-analysis: Not adjusted for confounders as the others and high risk of bias	
Chronic TMD (≥ 6 months), diagnosed via clinical examination (RDC/TMD criteria). Female subjects. N: 122 Non-painful stress urinary incontinence as controls. N: 122	1. Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups. 2. Multivariable logistic regression model (CI OR)	OR 1.53 [0.53–4.46] p 0.42 Association between subjects with chronic TMD and IC Small effect size OR 1.53α [0.29–7.93] p 0.61 Association between subjects with chronic TMD and IC Small effect size	
Chronic Orofacial pain (≥6 months), subjects per IASP and IHS criteria: secondary headaches (e.g., attributed to TMD), facial neuropathies, BMS, atypical odontalgia, systemic/metabolic headaches. Both sexes included. N: 174 No symptoms as controls N: 132	Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 1.56 [0.86–2.84] p 0.14 Association between subjects with chronic TMD and Gastritis Small effect size	OR 1.64 [1.12–2.41] p 0.01 Small effect size ⊕◯◯◯ Very low
Chronic painful TMD (≥6 months), as unique complaint, diagnosed with RDC/TMD criteria. Both sexes included. Comparator 1: TMD without migraine Comparator 2: TMD with migraine (diagnosed by structured questionnaire per ICHD-II) Subjects with chronic TMD N: 305 Control Healthy subjects N: 47	Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	1.TMD all OR 3.13 [1.28– 7.64] p 0.01 Association between subjects with chronic TMD and chronic Gastritis Small effect size 2.TMD with no Migraine OR 3.17 [1.07– 9.34] p 0.03 Association between subjects with chronic TMD and no Migraine and chronic Gastritis Small effect size 3.TMD with Migraine OR 3.1 [1.28–7.67] p 0.01 Association between subjects with chronic TMD and Migraine and chronic Gastritis Small effect size Excluded from Meta-analysis: focused exclusively on TMD, whereas meta-analysis includes broader orofacial pain diagnoses (not limited to TMD).	
Chronic Orofacial pain (≥6 months), subjects per IASP and IHS criteria: secondary headaches (e.g., attributed to TMD), facial neuropathies, BMS, atypical odontalgia, systemic/metabolic headaches. Both sexes included. N: 174 Control healthy subjects N: 132	1. Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups. 2. Multivariate linear regression analyses (β, CI)	OR 1.7 [1.08– 2.94] p 0.02 Association between subjects with chronic OFP and chronic Gastritis Small effect size β -0.012 [-0.08– 0.06] p 0.99π Association between subjects with chronic OFP and chronic Gastritis Small effect size	
Burning Mouth syndrome. Thorough clinical examination of the oral cavity. Detection of Smear Candida albicans detection was taken according to Budtz-Jorgensen. Measurement of salivary flow rate for evaluating xerostomia. Oral galvanism was measured. haematological screening for complete blood count with additional tests for blood glucose levels, serum ferritin and Helicobacter pylori antibodies. Both sexes included. N: 76 Sex and age matched controls N: 80	Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 2.7 [1.42– 5.40] p 0.002 Association between subjects with BMS and Gastritis Small effect size Excluded from Meta-analysis: focused exclusively on BMS, whereas meta-analysis includes broader orofacial pain diagnoses (not limited to BMS).	

	Factor	Studies (Study population/ Method/ RoB)	Condition definition / Exposure level
Association variables (Subjects with specific diagnosis vs. Controls without pain). Exposure to secondary disorders	8.Association between TMD and chronic IBD	Bucci 201816 (N total: 94) Cross-sectional (no temporal analysis) Clinical sample In total sample with (N:94), TMD was identified at the present time (N with TMD: 22, without TMD: 72). Prevalence of TMD was evaluated in subjects with IBD compared to healthy controls High risk of bias	TMD diagnosed by clinical exam per RDC/TMD. Subjects with CD: 7 YES, 21 NO; Subjects with UC: 6 YES, 13 NO; Controls: 9 YES, 38 NO
	9. Bidirectional association between chronic TMD and chronic GERD	Gharaibeh 201163 (N total: 120) Prospective Case-Control (no temporal analysis) Clinical sample In total sample with (N:120), chronic TMD was identified (N with chronic TMD: 33, without TMD: 87). Prevalence of TMD was evaluated in subjects with chronic GERD compared to healthy controls High risk of bias	TMD diagnosed by clinical examination (RDC/TMD criteria) in the last past 3 months. GERD subjects: 22 YES, 38 NO Control: 11 YES, 49 NO Comp. 1: Myofascial 19 YES, 41 NO Comp. 2: Joint pain 9 YES, 51 NO
		Li 201965 (N total: 3044) Cross-sectional (no temporal analysis) Clinical sample In total sample with (N:3044), chronic GERD was identified (N with chronic GERD: 193, without GERD: 2851). Prevalence of GERD was evaluated in subjects with chronic TMD compared to healthy controls Moderate risk of bias	GERD ≥3 months, diagnosed via validated GerdQ questionnaire and Montreal criteria. Subjects with TMD: 132 YES, 1390 NO Controls: 61 YES, 1461 NO
	10. Association between TMD and PCOS	Jedynak 202164 (N total: 126) Cross-sectional (no temporal analysis) Clinical sample In total sample with (N:126), TMD was identified (N with TMD: 94, without TMD: 32). Prevalence of TMD was evaluated in subjects with PCOS compared to healthy controls High risk of bias	TMD diagnosed through clinical examination according to DC criteria. Myofascial pain. Subjects with PCOS: 60 YES, 5 NO Controls: 34 YES, 27 NO
		Soydan 201471 (N total: 100) Cross-sectional (no temporal analysis) Clinical sample In total sample with (N:100), TMD was identified (N with TMD: 59, without TMD: 45). Prevalence of TMD was evaluated in subjects with PCOS compared to healthy controls High risk of bias	TMD symptoms such as preauricular pain, limited mouth opening, deviation or deflection during mouth opening, and joint sounds. Diagnosed by a clinician with Okeson's criteria. Subjects with PCOS: 47 YES, 7 NO Controls: 12 YES, 38 NO
		Yazici 202172 (N total: 75) Prospective case-control (no temporal analysis) Clinical sample In total sample with (N:75), TMD was identified (N with TMD: 25, without TMD: 50). Prevalence of TMD was evaluated in subjects with PCOS compared to healthy controls High risk of bias	TMD diagnosed through clinical examination according to RDC criteria Subjects with PCOS: 23 YES, 22 NO Controls: 2 YES, 28 NO
	11. Association between TMD and Endometriosis	Marciniak 202474 (N total: 163) Cross-sectional (no temporal analysis) Clinical sample In total sample with (N:163), TMD was identified (N with TMD: 80, without TMD: 83). Prevalence of TMD was evaluated in subjects with Endometriosis.	TMD in women taken from TMD Pain Screener questionnaire belonging to the DC/TMD criteria Subjects with Endometriosis: TMD YES 80 Subjects with Endometriosis: TMD NO 83
Dose-response/prognostic variables. Exposure to primary disorders	12. Association between IBS severity and TMD	Mobilio 202067 (N total: 82) Cross-sectional (no temporal analysis) Clinical sample The study compared subjects with TMD with healthy controls, analyzing the prevalence at the present time of IBS across different levels of exposure severity. High risk of bias	IBS-severity symptoms score counts of VAS. Exposure 1. No pain (< 75 points) Exposure 2. Mild pain (75 – 175 points) Exposure 3. Moderate pain (175 – 300 points) Exposure 4. Severe pain (> 300)
	13. Association between IBS Severity and Chronic TMD	R. Ohrbach 201168 (N total: 1802) Nested case-control study within a prospective cohort (IBS before chronic TMD) Community or population-based The study compared subjects with chronic TMD to healthy controls, analyzing the prevalence of previous IBS across different levels of exposure severity. Low risk of bias	IBS diagnosed with self-reported questionnaires including ROME III. Counts of 10 IBS symptoms severity association to chronic TMD. Exposure 1. Low severity (< 2 counts) Exposure 2. Moderate severity (3 – 5 counts) Exposure 3. High severity (> 6 counts)
	14. Association between IBS and TMD duration	Dahan 201513 (N total: 180) Cross-sectional (no temporal analysis) Clinical sample The study evaluated subjects with myofascial TMD compared to subjects with non-myofascial TMD, analyzing the correlation between the presence of IBS and the duration of TMD High risk of bias	IBS (YES NO) association to the TMD pain duration. IBS diagnosed with self-reported questionnaires (ROME III). Subjects with TMD 51 YES 129 NO
	15. Association between IBS and TMD severity	Dahan 201513 (N total: 180) Cross-sectional (no temporal analysis) Clinical sample The study evaluated subjects with myofascial TMD compared to subjects with non-myofascial TMD, analyzing the correlation between the presence of IBS and the severity of TMD. High risk of bias	IBS (YES NO) association to the TMD pain intensity IBS diagnosed with self-reported questionnaires (ROME III). Subjects 51 YES 129 NO

*Multivariate Random forests adjusted for study site, imputing for missing data. ¥ Rate ratio represents relative increase in incidence rate of TMD relative to reference group. Calculated using Cox proportional hazards regression model controlling for study site (categorical variable, 4 levels). With additional adjustment for age in years, gender (categorical, 2 levels), race/ethnicity (categorical, 2 levels), and lifetime U.S. residence (categorical, 2 levels) and with inclusion of imputed TMD rates for subjects lost to follow-up and imputed data for values missing at baseline. £ Multivariate logistic regression model. TMD as the outcome to model all demographic and clinical variables simultaneously (N=3,874,625; those with any missing variabl es were dropped from analysis). Adjusting for different ages, races, marital status, migraines, tension type headache, headache, IBS, depression, posttraumatic sindrome, anxiety, alcohol use, other substance use. § Putative risk factor is the main explanatory variable and study site is the covariate Fully-adjusted effects were computed in logistic regression models that additionally include covariates of age group, gender, and race/ethnicity. §§ Factores included: chronic widespread pain; chronic fatigue. Adjusted for age and gender. a Odds ratios adjusted for age and gender where appropriate. ‡ Crude model including all comorbidities and potential confounders. Adjusted by sex, age, and employment status. Reference group non miofascial. α Multivariate logistic regression was carried out to estimate adjusted odds ratios for the presence of comorbid disorders, comparing the IC/BPS and SUI groups, with 95% confidence intervals. Adjusted for confounders IBS, Multiple chemical sensitivities, Headache, myofascial pain, Fibromyalgia. π The final multivariate model included the following factors: age, functional score, otorhinolaryngologic diseases, number of non-craniofacial pain areas, sleep quality, digestive complaints, number of trigger points, number of remaining teeth, xerostomia score, numbness intensity, and bruxism. ± Multivariate logistic regression model adjusting for age, sex, residence, body mass index, education, marital status, smoking, alcohol, tea, coffee, teeth clenching or grinding during sleep or waking hours. ∞ COX regression. was used to estimate RR adjusted for potential confounders. Age. + Within-Pair Effects. Mixed-effects logistic regression models accounting for twin pairs and adjusted for age. # Adjusted by age and confounders. A confounding RR ≥ 0 reflects the extent that familial confounding factors account for the individual- level association, while a value of 1 suggests that confounding factors have no effect on the individual- level association **Multivariate linear regression analyses were done considering the confounding were performed. Complete Model including all confounder. Adjusted for sex, TMD subgroups, marital status, work status and psychological history. ££ Fully-adjusted effects were computed in logistic regression models that additionally include covariates of age group, gender, and race/ethnicity. Note. This summary table presents the main results, organized into three categories: (1) Predictor / prognosis variables, exposures assessed before TMD onset or chronic TMD, ensuring temporality between exposure and outcome; (2) Association variables, based on cross-sectional analyses without a defined temporal relationship; and (3) Dose-response/prognostic variables, which assess how exposure intensity or severity influences the course of OFP or TMD. Each category is further divided into exposures to primary disorders and secondary disorders. Columns in the table include: the exposure or factor studied, the included studies, exposure definitions, Subjects diagnostic criteria, statistical methods, individual results, meta-analytical findings (if available), and the certainty level of the evidence. Effect sizes are interpreted using thresholds adapted from Cohen for OR and RR: ≤ 1.68: irrelevant, 1.69–3.47: small, 3.48–6.71: moderate, ≥ 6.72: large. For HR we followed Deepti et al.: ≤ 1: no risk, 1.2–1.5: moderate risk, 1.5: high risk or large effect. For β coefficients, effect sizes were interpreted based on the scale of the outcome variable and their clinical or practical relevance. For example, in outcomes such as pain intensity measured by a numeric rating scale rating 0–10, a β ≥ 0.5 was interpreted as minimally clinically important, while β ≥ 1.0 indicated a clinically relevant change. In the case of pain duration measured in years, changes exceeding 1 year were considered substantial when the average baseline duration was around 10 years. Abbreviations: TMD: Temporomandibular disorders; OFP: Orofacial pain; IBS: Irritable bowel syndrome; IC: Interstitial cystitis; PCOS: Polycystic Ovary Syndrome; IBD: Inflammatory bowel disease; GERD: Gastroesophageal reflux disease; odds ratio: OR; risk ratio: RR; hazard ratio: HR; regression coefficients: β

Subjects' diagnosis definition / Exposure level	Statistical analysis	Individual results 95% CI	Results in meta-analysis / Certainty
IBD (≥6 months) diagnosed by clinical, endoscopic and radiologic criteria. Diagnosed through clinical, endoscopic and radiologic. Comparator 1. Crohn's disease (CD). Both sexes included. N: 28 Comparator 2. Ulcerative colitis (UC) N: 19 Control Healthy subjects N: 47	Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	IBD OR 1.61 [0.61–4.24] p 0.33 Association between subjects with chronic IBD and TMD. Small effect size Comp 1 (CD) OR 1.40 [0.35–4.32] p 0.55 Association between subjects with chronic CD and TMD. small effect size Comp 2 (UC) OR 1.94 [0.58–6.53] p 0.27 Association between subjects with chronic UC and TMD. Small effect size	No meta-analysis ⊕◯◯◯ Very low
GERD ≥3 months, diagnosed based on clinical symptoms and response to acid-suppressive therapy. Both sexes included. N: 60 Control Healthy subjects N: 60	Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 2.57 [1.11–5.96] p 0.02 Association between subjects with chronic GERD and TMD Small effect size	OR 2.71 [1.92: 3.82] p <0.001 Small effect size ⊕⊕⊕◯ Moderate
Chronic TMD ≥6 months, diagnosed using RDC/TMD criteria. Both sexes included. N: 1522 Matched healthy controls N: 1522	1. Crude logistic regression model (OR, CI), based on "Yes/No" distribution in exposed vs. non-exposed groups. 2.multivariable logistic regression (OR, CI)	1. OR 2.61 [1.88–3.63] p <0.0001 Association between subjects with chronic TMD and GERD Small effect size 2. OR 2.74± [1.88–3.98] Association between subjects with chronic TMD and GERD Small effect size	
Clinical or biochemical (or both) hyperandrogenism, hyperinsulinemia, oligomenorrhoea or anovulation, and polycystic ovaries, according to Rotter-dam criteria. Female subjects. N: 65 Control healthy subjects N: 61	Crude logistic regression model (OR, 95% CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 9.52 [3.35–27.03] p <0.0001 Association between subjects with PCOS and TMD Large effect size	OR 12.81 [5.39–30.44] <0.001 Large effect size ⊕⊕⊕◯ Moderate
PCOS was diagnosed through clinical examination by an endocrinologist. Female subjects. N: 50 Healthy control N: 50	Crude logistic regression model (OR, 95% CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 19.45 [6.94–54.44] p <0.0001 Association between subjects with PCOS and TMD. Large effect size Excluded from analysis: Accepted but not validated diagnostic criteria	
PCOS based on ESHRE/ASRM criteria: anovulation, signs of hyperandrogenism, or polycystic ovarian morphology observed via ultrasound. Female subjects. N: 45 Healthy control N: 30	Crude logistic regression model (OR, 95% CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 14.63 [3.10–68.89] p <0.0007 Association between subjects with PCOS and TMD. Large effect size	
Confirmation of a diagnosis of endometriosis made by a medical doctor. Female subjects. N: 163	Crude logistic regression model (OR, 95% CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	OR 0.92 [0.60–1.43] p 0.73 Association between subjects with Endometriosis and TMD. Small effect size	No Meta analysis ⊕◯◯◯ Very low
Presence of at least one TMD diagnosis according to RDC criteria. Both sexes included. N: 47 Control Healthy subjects. N: 35	Crude logistic regression model (OR, 95% CI), based on "Yes/No" distribution in exposed vs. non-exposed groups.	Exposure 1. OR 0.14 [0.04–0.48] p < 0.001 Small effect size Exposure 2. OR 2.41 [0.45–12.75] p < 0.29 Small effect size Exposure 3. OR 4.45 [0.91–21.84] p < 0.065 Moderate effect size Exposure 4. OR 11.2 [0.6–204.37] p < 0.1 Very large effect size	No meta-analysis ⊕⊕⊕◯ Moderate
TMDs chronic (≥ 6 months). Diagnosed following RDC/TMD criteria. ≥5 days/month of pain in TMD locations specified by examiner and examiner findings of arthralgia or myalgia. Both sexes included. N:185 Control Healthy subjects. N: 1601	Logistic Regression models (CI OR)	Exposure 1. OR 1.3££ [0.8: 2] <0.0001 Small effect size Exposure 2. OR 2.6££ [1.7: 3.9] <0.0001 Moderate effect size Exposure 3. OR 3.8££ [2.2: 6.3] <0.0001 Moderate effect size	No meta-analysis ⊕◯◯◯ Very low
TMD (mean duration: 6.3 ± 0.6 years), diagnosed using RDC/TMD criteria. Both sexes included. Comp. 1: m-TMD (myofascial pain only) Comp. 2: n-TMD (non-myofascial pain). For TMD pain DURATION was assessed by ASKING How long have you had pain in the face? N: 180	Multivariate linear regression analyses were performed with TMD duration as the dependent variable (CI and β)	TMD all β 2.24** [-0.41: 4.90] p < 0.1 Large effect size m TMD β 3.14** [-0.04: 6.25] p < 0.05 Very large effect size n TMD β 0.69** [-5.36: 6.74] p < 0.8 Moderate effect size	No meta-analysis ⊕⊕⊕◯ Moderate
TMD pain intensity assessed by the Numerical Pain Rating Scale. Diagnosed using RDC/TMD criteria. Both sexes included. Comp. 1: m-TMD (myofascial pain only) Comp. 2: n-TMD (non-myofascial pain). For TMD pain DURATION was assessed by ASKING How long have you had pain in the face? N= 180	Multivariate linear regression analyses were performed with TMD severity as the dependent variable (CI and β)	TMD all β 0.10** [-0.73: 0.94] p < 0.81 Moderate effect size m TMD β 0.05** [-0.97: 0.87] p < 0.44 Small effect size n TMD β 0.75** [-1.29: 2.79] p < 0.8 Moderate effect size	No meta-analysis ⊕◯◯◯ Very low

Table 2. General study characteristics

Study characteristics ($N = 22$)		*n*
Country	E.E.U.U[56,61,66,68,70]	5
	Canada[13,58]	2
	Italy[16,62,67]	3
	UK[73]	1
	Poland[64,74]	2
	Jordan[63]	1
	Brazil[57,59,69]	3
	China[65]	1
	Taiwan[61]	1
	Turkey[71,72]	2
	Croatia[75]	1
Publication date	From 2000 to 2010[56,63,76,73,75]	5
	From 2011 to 2020[71,13,16,57,58,60–62,65,67–70]	13
	From 2021[59,64,72,74]	4
Study design	Longitudinal[59,61,63,66,70,72,74]	7
	Nested case control[68]	1
	Cross sectional (rest of studies)	14
Ethical committee approval	Yes	21
	No	0
	Not reported[66]	1
Funding	Not reported[71,57,62,63,69,75]	6
	No funding[59,60,64,67,74]	5
	Government[56,61,65,66,68,73]	6
	Foundation [13,16,58,72]	4
	Academic[70]	1
Sampling method	Convenience[71,13,16,56–58,60–63,65–67,69,70,71–75]	19
	Consecutive[59,64]	2
	Random[68]	1
Gender	Female[71,64,66,72,74]	5
	Both mixed (rest of the studies)	17
Language	English (all)	22
	Other	0
Patients diagnostic main condition	TMD chronic[13,57,58,61,65,58]	6
	TMD first-onset[66,70]	2
	TMD general[56,67]	2
	OFP[59,69,73]	3
	BMS[75]	1
	IBS[62]	1
	IBD[16]	1
	PCOS [71,64,72]	3
	GERD[63]	1
	IC[60]	1
	Endometriosis[74]	1
Diagnostic tools for Orofacial pain	DC/TMD[23,74]	2
	RDC/TMD [13,16,56–89,60,62,63,65,66,67,68,70,72]	14
	OFP according to the IASP[59,69]	2
	OFP through validated self-reported questionnaire (Classification Questionnaire for Orofacial Pain)[73]	1
	TMD Defined by experts. Defined as ICD[61]	1
	TMD by Okeson's Muscle clinical examination form[71]	1
	OFP secondary to BMS through a clinical examination ruling out local and systemic causes[75]	1

TMD – temporomandibular disorders – chronic, first-onset, or general; OFP – orofacial pain; BMS – burning mouth syndrome; IBS – irritable bowel syndrome; IBD – inflammatory bowel disease (IBD); PCOS – polycystic ovary syndrome; GERD – gastroesophageal reflux disease; IC – interstitial cystitis; DC/TMD – Diagnostic Criteria for TMD; RDC/TMD – Research Diagnostic Criteria for TMD; IASP – International Association for the Study of Pain criteria; ICD – International Classification of Diseases.

Temporality-oriented studies

In the 1st group, we identified 2 types of studies: 2 prospective longitudinal studies by Lim et al.[66] and Sanders et al.,[70] and a nested case–control study by Ohrbach et al.[68] based on Sanders' cohort, which used a prospective analysis.[68] These studies diagnosed TMD using the RDC/TMD. Ohrbach et al.[68] and Sanders et al.[70] diagnosed IBS using Rome III, and analyzed the risk of first-onset TMD and its chronification in individuals with IBS. Lim et al. used a self-report instrument based on RDC/TMD to analyze the risk of first-onset TMD in individuals with dysmenorrhea.[66] All studies included comparison groups of participants without TMD, enabling risk estimation by comparing exposed and non-exposed individuals. In addition, the prospective population-based study conducted by Aggarwal et al. examined the onset of chronic OFP, including TMD, over a 2-year follow-up.[73] The study assessed how co-occurring pain conditions, such as IBS diagnosed according to the Rome II criteria, influenced the development of OFP.[73]

Predictor or prognosis variables. Exposure to primary disorders

Based on the studies mentioned above,[66,68,70,73] we could group the variables used to analyze the effects of the predictive and prognostic relationships related to primary pain disorders. Specifically, they examined: (1) the likelihood of first-onset TMD among subjects with IBS[70]; (2) the likelihood of first-onset TMD among subjects with dysmenorrhea[66]; and (3) the likelihood of chronic OFP among subjects with IBS.[68,73]

In the reviewed literature, limited longitudinal studies precluded stratified meta-analyses by diagnosis (i.e., TMD, BMS) in relation to prognostic factors (e.g., IBS, IBD, gastritis); the only association permitting meta-analytic inference was IBS in relation to chronic OFP (3). This was based on 2 large studies with comparable populations, validated diagnostic criteria for OFP and IBS, and similar adjustments for confounders, showing a consistent trend toward increased likelihood of developing TMD, BMS or other OFP diagnoses among those with prior IBS symptoms. The meta-analysis yielded a pooled *OR* of 2.32 (95% *CI*: 1.43–3.76), indicating more than a twofold increase in odds for those exposed (Fig. 3).

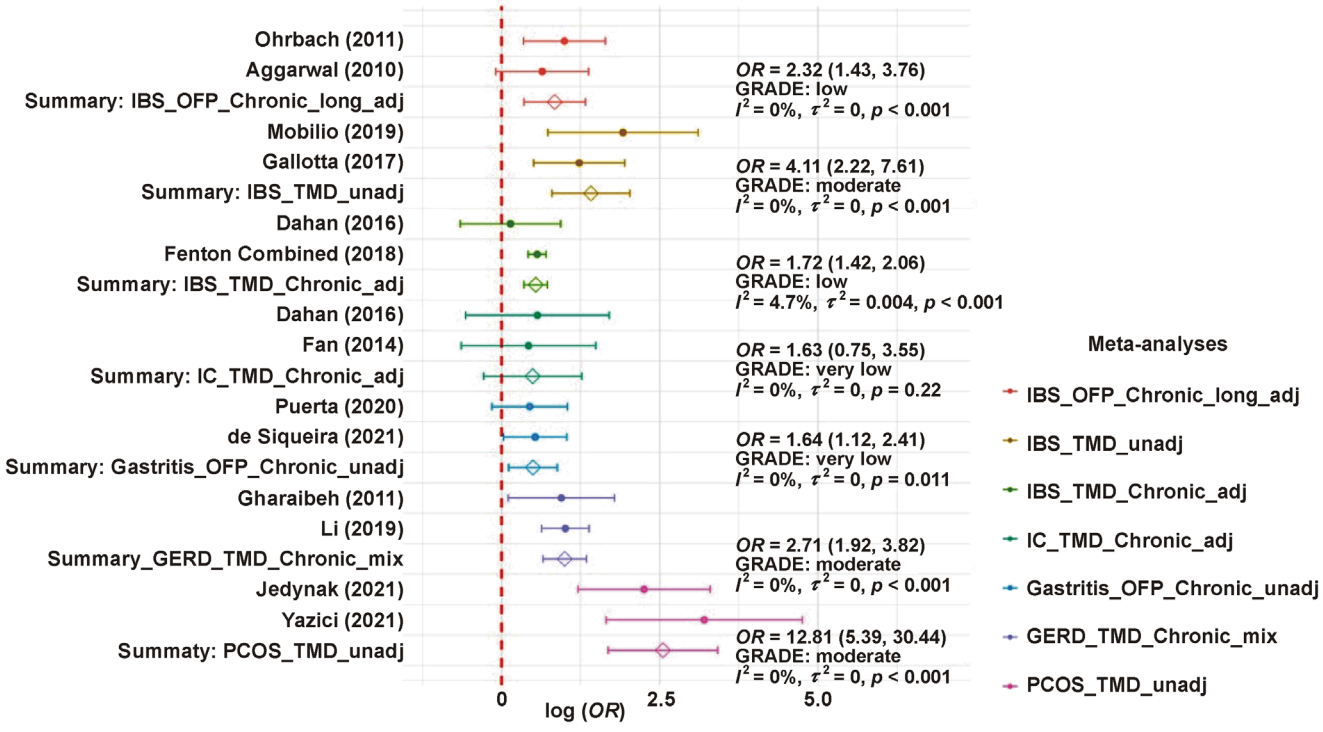


Fig. 3. Forest plot of individual studies and the pooled estimates for 5 meta-analyses assessing the likelihood of comorbid conditions in temporomandibular disorders (TMD)

Each dot represents an individual study with its corresponding 95% *CI*. Diamonds indicate the pooled effect size for each meta-analysis using a random-effects model. The vertical dashed red line represents the null value (log (*OR*) = 0). The GRADE (Grading of Recommendations Assessment, Development and Evaluation) certainty levels are displayed next to the summary estimates. IBS_OFP_Chronic_long_adj: Prognostic association between IBS and chronic OFP; IBS_TMD_unadj: Bidirectional association between TMD and IBS; IBS_TMD_Chronic_adj: Association between IBS and chronic TMD; IC_TMD_Chronic_adj: Association between IC and chronic TMD; Gastritis_OFP_Chronic_unadj: Association between gastritis and chronic OFP; GERD_TMD_Chronic_mix: Bidirectional association between chronic TMD and chronic GERD; PCOS_TMD_unadj: Association between TMD and PCOS. All analyses were conducted in comparison to healthy controls or individuals without myofascial pain. The suffix in each factor name (e.g., _adj, _unadj, _mix) indicates the type of statistical model used: _adj include only the adjusted estimates; _unadj indicates only the unadjusted estimates; and _mix denotes the inclusion of both the adjusted and unadjusted estimates.

The evidence indicates moderate certainty that IBS is associated with increased likelihood of first-onset TMD (1) and low certainty that it is associated with chronic OFP (3). However, the evidence linking dysmenorrhea to chronic OFP (2) has low certainty due to study limitations (Table 1; supplementary material S4).

Cross-sectional associative studies

This group consists mostly of most cross-sectional studies.[13,16,56–58,60,62,64,65,67,69,71,74,75] We included the nested case–control study by Ohrbach et al.,[68] and the case–control studies by de Siqueira et al.,[59] Gharaibeh et al.[63] and Yazici et al.,[72] as they incorporated a cross-sectional analysis of the data. Most studies diagnosed TMD using RDC/TMD,[13,16,57,58,60,62,63,65,67,68,72] while Jedynak et al.[64] and Marciniak et al.[74] applied DC/TMD. Fenton et al. identified TMD cases based on 2 positive clinical examinations within 18 months, using the ICD definitions.[61] Soydan et al. diagnosed TMD using a structured form, Okeson's questionnaire and criteria.[71] Puerta et al.[69] and di Siqueira et al.[59] focused on OFP diagnosis, including TMD, BMS, headaches, and neuropathies, based on the IASP[76] and the IHS[31] criteria. In contrast, Brailo et al. diagnosed OFP secondary to BMS through a clinical examination, ruling out local and systemic causes.[75]

From the analyzed studies, most included both men and women. However, some studies focused only on women-related issues like menstrual pain, endometriosis and PCOS.[64,66,71,72,74] Fenton et al. showed results separately for men and women.[61] To align with other studies that presented combined estimates, we merged the male and female data using a fixed-effects meta-analysis. A fixed-effects approach was appropriate here, as both estimates originated from the same study, with the same variables and methodology, and the only difference was sex. We applied the inverse-variance method to log *OR*s and their *SE*s to obtain the pooled estimate.

Association variables. Exposure to primary and secondary disorders

From these studies performing cross-sectional analyses, we could extract variables to analyze associative relationships between TMD and other COPCs. Specifically, they examined: (4) a bidirectional association between TMD and IBS[62,67]: assesses studies in which either TMD or IBS was the primary diagnosis or an associated condition, evaluating their co-occurrence; (5) the association between IBS and chronic TMD[56,58,61]: evaluates the presence of IBS in individuals with a clinical diagnosis of chronic TMD; (6) the association between IC and chronic TMD[56,58,60]: explores the presence of IC in individuals diagnosed with chronic TMD; (7) the association between gastritis and chronic OFP[57,59,69,75]: examines the presence of gastritis in individuals diagnosed with OFP; (8) the association between TMD and chronic IBD[16]: investigates the presence of TMD in individuals with a diagnosis of chronic IBD; (9) a bidirectional association between chronic TMD and chronic GERD[63,65]: assesses studies in which either chronic TMD or GERD is the main or associated diagnosis, examining their mutual relationship; (10) the association between TMD and PCOS[64,71,72]: evaluates the presence of TMD in individuals diagnosed with PCOS; and (11) the association between TMD and endometriosis[74]: evaluates the presence of TMD in individuals diagnosed with endometriosis. Variables 1 to 4 were primarily related to primary pain disorders, whereas variables 5 to 8 were associated with secondary pain due to the underlying organic pathology.

The reviewed studies reported varied associations between TMD (or chronic TMD) and comorbidities, with effect sizes from very small to large. The estimates, RoB assessments and study characteristics are presented in Table 1, and meta-analytical forest plots in Fig. 3. Notably, the strongest, most consistent associations were for functional comorbidities, the association between IBS and TMD (4) being the most prominent (*OR*: 4.11; 95% *CI*: 2.22–7.61; $p < 0.001$).[62,67] Regarding organic conditions, the PCOS–TMD association (10) showed a strong effect (*OR*: 12.81; 95% *CI*: 5.39–30.44; $p < 0.001$),[64,72] while the GERD–chronic TMD association (9) presented a small effect, approaching moderate (*OR*: 2.71; 95% *CI*: 1.92–3.82; $p < 0.001$).[63,65]

For primary disorders, the certainty of evidence was generally low, with moderate certainty for the associations between GERD and PCOS and TMD (9 and 10). Most associations, including IBS (4) or gastritis (7), have very low to low certainty due to small samples, cross-sectional designs and a high RoB. For secondary disorders, the certainty of evidence was mostly low to very low, with moderate certainty only for GERD and PCOS (Table 1; supplementary material S4).

Dose–response variables. Exposure to primary disorders

From the cross-sectional analyses we also extracted the variables used to examine the graded relationship between IBS and different dimensions of TMD, such as pain intensity, pain duration or pain location. Specifically, they examined: (12) the association between IBS severity and TMD diagnosis,[67] evaluating whether increasing levels of IBS symptom severity were associated with the likelihood of having at least one type of TMD diagnosis; (13) the association between IBS severity and chronic TMD,[68] assessing whether greater IBS severity predicts the presence of chronic TMD; (14) the association between IBS and TMD pain duration,[13] investigating whether the presence of IBS is linked to longer duration of TMD-related facial pain, stratified by myofascial and non-myofascial diagnostic subtypes; and (15) the association between IBS

and TMD pain severity,[13] examining whether individuals with IBS report higher TMD pain severity as compared to those without IBS, also stratified by the TMD subtype.

In the association between IBS severity and TMD (12), the data indicated an increase in the likelihood of TMD diagnosis with higher IBS severity levels, following an exponential pattern in the odds across severity categories. For the association between IBS severity and chronic TMD (13), the odds of chronic TMD increased progressively with higher IBS severity. Individuals with IBS presented longer durations of TMD pain, particularly in the myofascial subtype (14). In relation to TMD pain severity (15), IBS was associated with slightly higher pain intensity scores, with no substantial differences between the diagnostic subtypes. These exposure variables and stratified outcomes are presented in Fig. 4.

The certainty of evidence was moderate for the graded association between IBS severity and general TMD outcomes, including pain duration. For chronic TMD and TMD pain intensity, the certainty was very low due to small samples and methodological limitations (Table 1; supplementary material S4).).

Sensitivity analysis of the conducted meta-analyses

The LOO analysis indicated varying levels of stability across the meta-analyses. In the assessment of the likelihood of chronic OFP among individuals with IBS, a low level of instability was noted, attributed to the *CI* reported by Aggarwal et al.,[73] which narrowly encompassed zero. However, upon aggregating the 2 studies,[68,73] no heterogeneity was observed, and the effects remained consistent. Consequently, we retained this study in the meta-analysis.

In the gastritis and chronic OFP model, including 2 studies[57,75] introduced moderate heterogeneity, though it did not change the overall direction of the effect. While the *CI*s became more consistent across iterations, the heterogeneity justified excluding these studies to enhance model homogeneity and robustness.

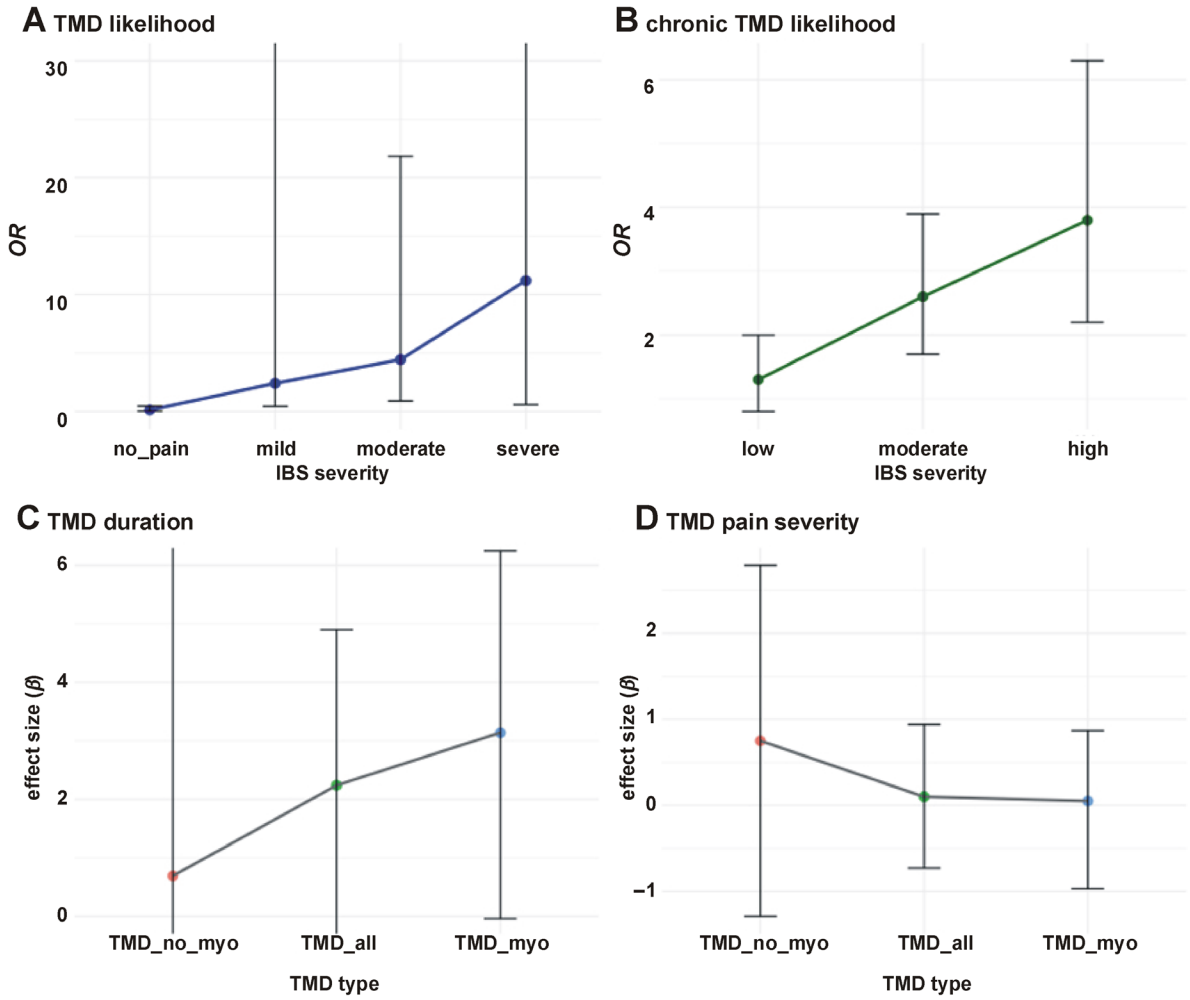


Fig. 4. Slope plots of dose–response gradients between irritable bowel syndrome (IBS) severity and prognostic outcomes of temporomandibular disorders (TMD)

A – likelihood of developing TMD across increasing IBS severity levels; B – prognostic likelihood of developing chronic TMD in relation to IBS severity levels; C – association between IBS and TMD duration, stratified by the clinical TMD subtype (TMD_no_myo – free of myofascial pain; TMD_all – all types of TMD; TMD_myo – reported myofascial pain alone); D – association between IBS and TMD pain severity, stratified by the clinical TMD subtype. The effect estimates are shown as *OR* or *β* (regression coefficients) with 95% *CI*s. Connecting lines highlight trends across exposure levels.

In the IBS and chronic TMD model, high heterogeneity (I^2 > 70%), and significant variation in *OR* and *CI* indicated the instability of estimates. To improve consistency to I^2 = 4.7%, we excluded the study with the highest RoB and the lack of confounder adjustment.[56]

A similar approach was applied to the IC and TMD model. Excluding the study by Aaron et al.[56] improved stability by reducing heterogeneity to I^2 = 0.0% and minimizing the methodological variation influence.

For the model assessing the association between TMD and PCOS, we directly excluded the study by Soydan et al., as it used a clinically accepted diagnostic criterion lacking formal validation.[71]

In the meta-analysis of the bidirectional association between chronic TMD and chronic GERD, the 2 available studies differed in methodological rigor, as one adjusted for confounders and had a larger sample,[65] while the other did not.[63] Despite this, both showed nearly identical effect sizes, and the pooled analysis revealed no heterogeneity. Therefore, we retained the meta-analysis and reported it as a mixed model.

Following the LOO analysis, all models exhibited stability and homogeneity, with I^2 values ranging from 0.0% to 4.7%, indicating minimal variation in the pooled estimates across iterations. The comprehensive results of the LOO analysis are presented in Table 3.

Discussion

Summary of findings

This review evaluated evidence regarding the associations between COPCs and OFP, focusing on TMD, identifying the strength, direction and temporal characteristics of these associations.

We found consistent evidence of positive associations between COPCs and OFP, particularly for IBS, GERD and PCOS. Although longitudinal studies were limited, prognostic meta-analysis indicated with low certainty that IBS increases the likelihood of chronic OFP (*OR*: 2.32; 95% *CI*: 1.43–3.76), including TMD and BMS. Irritable bowel syndrome showed a temporal relationship with first-onset TMD and chronic OFP.

Other meta-analyses based on cross-sectional data reflected associative rather than predictive relationships. The pooled estimates indicated moderate to large effects: IBS was associated with chronic TMD (*OR*: 1.72; 95% *CI*: 1.42–2.06), GERD with chronic TMD (*OR*: 2.71; 95% *CI*: 1.92–3.82), and PCOS showed the strongest association with TMD (*OR*: 12.81; 95% *CI*: 5.39–30.44). However, these latter associations (GERD and PCOS) should be interpreted with caution, as they are based on unadjusted analyses that did not account for potential confounding

Table 3. Leave-one-out meta-analysis summary

MetaGroup	Study excluded	*OR* without	95% CI	I^2	Change *OR* (%)
Risk of chronic Orofacial pain among subjects with IBS	R Ohrbach (2011)	1.90	(0.91, 3.96)	0.0	–17.94
	Aggarwal (2010)	2.70	(1.41, 5.15)	0.0	16.61
Association between Gastritis and Orofacial Pain	Puerta (2020)	2.19	(1.48, 3.26)	11.8	10.93
	Contreras (2018)	1.86	(1.33, 2.59)	0.0	–6.16
	De Siqueira (2021)	2.21	(1.42, 3.43)	17.0	11.54
	Brailo (2006)	1.81	(1.28, 2.58)	0.0	–8.30
Association between IBS and Chronic TMD	Dahan (2016)	3.21	(0.75, 13.80)	79.4	49.40
	Aaron (2000)	1.72	(1.42, 2.06)	4.7	–20.29
	Fenton Combined (2018)	2.80	(0.42, 18.64)	83.1	30.31
Bidirectional association between TMD and IBS	Mobilio (2020)	3.41	(1.66, 7.01)	0.0	–17.00
	Gallota (2017)	6.82	(2.08, 22.37)	0.0	66.00
Association between IC and chronic TMD	Dahan (2016)	2.17	(0.53, 8.8)	20.5	18.79
	Aaron (2000)	1.63	(0.75, 3.55)	0.0	–10.48
	Fan (2014)	2.25	(0.71, 7.15)	5.2	23.06
Association between TMD and PCOS	Jedynak (2021)	20.91	(8.87, 49.31)	0.0	37.32
	Soydan (2014)	12.81	(5.39, 30.44)	0.0	–15.90
	Yazici (2021)	13.67	(6.57, 28.46)	0.0	–10.20
Bidirectional association between chronic TMD and chronic GERD	Gharaibeh (2011)	2.74	(1.88, 3.99)	0.0	0.67
	Li (2019)	2.62	(1.07, 6.41)	0.0	–3.74

TMD – Temporomandibular disorders; OFP – Orofacial pain; IBS – Irritable bowel syndrome; IC – Interstitial cystitis; PCOS – Polycystic Ovary Syndrome; IBD – Inflammatory bowel disease; GERD – Gastroesophageal reflux disease; *OR* – odds ratio; *CI* – confidence intervals. Leave-one-out (jackknife) sensitivity analysis was conducted for each meta-analysis by recalculating the *OR* after omitting one study at a time. The table reports the revised *OR*, 95% *CI*, heterogeneity (I^2), and percentage change in *OR*.

variables. Overall, since most studies did not adequately control for confounders, the certainty of these associations was downgraded to low or very low. This included the remaining conditions (IC, gastritis, IBD, and endometriosis), where the evidence was further limited by small sample sizes and a high RoB.

Literature gaps persist. While studies used validated criteria like DC/TMD or ICOP-1, many were excluded due to inadequate diagnostic standards. Most included studies were cross-sectional, lacked confounder adjustment and showed heterogeneity. Limited longitudinal data restricted causal inferences.

These limitations highlight the need for prospective studies with standardized diagnostics, confounder control, and the exploration of shared pathways like central sensitization and inflammation.

Comparison with previous literature

This review corroborates previous narrative reviews examining the relationship between OFP and COPCs. Da-Cas et al. suggested that painful TMD may be nociplastic, given its overlapping characteristics with other COPCs, such as fatigue, sleep disturbances and central sensitization, leading to pain-related disability.[19] Our review extends these observations by addressing a distinct research question on the putative risk factors involved in TMD onset and by quantitatively exploring the magnitude of associations between OFP/TMD and systemic conditions.

Similarly, Kleykamp et al. reviewed comorbidities in TMD patients and reported a higher prevalence of several COPCs, including IBS, myofascial pain and fibromyalgia, as compared to non-TMD individuals,; however, their analysis was limited to cross-sectional evidence.[9] In contrast, our meta-analysis integrates both cross-sectional and prospective longitudinal data, providing a more comprehensive framework to explore not only co-occurrence, but also potential etiopathogenic links between these conditions.

Recent studies have highlighted immune activation and the role of cytokines in IBS, particularly regarding pain mechanisms.[77,78] Irritable bowel syndrome is associated with altered signaling between immune cells and sensory neurons in the gut, driving chronic pain.[78] These inflammatory mediators and nociplastic pathways extend beyond the gut; cytokines can enhance neuronal excitability and promote central sensitization in the trigeminal system, contributing to hyperalgesia and orofacial muscle pain in TMD. Immunological evidence from IBS supports the hypothesis that generalized muscle pain sensitivity in TMD is mediated by nociplastic mechanisms, consistent with reviews on myofascial trigger points and nociplastic pain, where the induced trigger points produce persistent hyperalgesia and central sensitization without evident tissue damage.[78]

Saczuk et al. reviewed the relationship between IBS and TMD, highlighting mechanisms like autonomic dysfunction and systemic inflammation.[23] Our findings complement their work by confirming the association and providing effect sizes, supporting IBS as a contributor to TMD. Basic research shows that estradiol and stress can induce visceral and cutaneous hypersensitivity with central sensitization, predisposing individuals to OFP, and explaining the comorbidity between IBS and TMD.[79] Moisset et al. focused specifically on patients diagnosed with BMS, demonstrating its co-occurrence with TMD, fibromyalgia and IBS, and positioning BMS within the COPC spectrum.[20] Our review expands on this perspective by including BMS within the broader category of OFP, considering it another phenotype of nociplastic pain that shares common underlying mechanisms with TMD and other COPCs.

The effect sizes observed between TMD and GERD in our synthesis are noteworthy. These magnitudes exceed those seen for primary pain disorders, suggesting that GERD, through the mechanisms of tissue damage, inflammation and altered pH, may accelerate the peripheral and central sensitization underlying chronic OFP. This aligns with Manfredini's multidimensional framework,[80] which describes how GERD, via sleep disruption and parafunctional activities, creates conditions for chronic OFP. Li et al. reported that GERD was more prevalent in BMS patients, suggesting that reflux-induced pH alterations and sensory disturbances in the orofacial region may predispose to chronic OFP.[81]

The association between TMD and PCOS was robust. Evidence shows TMD is more prevalent and severe in women with PCOS, due to systemic inflammation and altered sex hormone-mediated pathways.[72] Supporting this, Sun et al. observed that adolescents with untreated PCOS and anterior disc displacement had greater condylar resorption and reduced bone regeneration after disc repositioning surgery.[82]

This progression appears in our meta-analytical forest plot, with primary conditions shown in the upper section and secondary conditions in the lower section, displaying increasing effect sizes from top to bottom. These observations suggest that diverse COPCs, through the overlapping inflammatory, hormonal and neural pathways, converge on shared nociplastic mechanisms that sustain chronic OFP.

Mechanistic considerations

The findings align with the literature framing TMD as nociplastic pain conditions, where pain stems from altered central nociceptive processing rather than peripheral pathology. Svensson characterized TMD as a model of nociplastic pain, emphasizing central sensitization, impaired pain modulation and pain facilitation prevalent in other COPCs.[83]

Expanding on this idea, various biological fields seem to interact in the pathophysiology of TMD and associated COPCs. Hormonal, inflammatory/immune, and

neuroplastic processes come together to maintain chronic pain and clarify the connections between gastrointestinal, gynecological and orofacial pain conditions.

This framework aligns with central sensitivity syndromes – the overlapping conditions including TMD, IBS, fibromyalgia, chronic headache, and pelvic pain syndromes that share persistent pain despite differing anatomical locations. The model proposes these disorders are unified by central sensitization, with subjects showing lowered sensory thresholds and heightened responsiveness to nociceptive input.[84] Subjects present with common psychosocial features like depression, anxiety, fatigue, and cognitive difficulties, which interact with biological processes to sustain pain.[84] Thus, the comorbidity between TMD and other COPCs reflects not isolated phenomena, but shared central mechanisms and vulnerability profiles within a biopsychosocial context.

Supporting this mechanistic framework, neuroimaging research provides insight into central sensitization. Lam et al. examined the cortical footprint of COPCs by comparing females with TMD-related chronic pain and fibromyalgia with and pain-free controls, using structural magnetic resonance imaging (MRI).[85] They found cortical and subcortical alterations in the regions involved in pain modulation, affective-emotional processing, and sensorimotor integration. These changes correlated with pain distribution, pain catastrophizing and affective distress, supporting that COPCs share neuroplastic brain alterations affecting sensory-discriminative and affective-cognitive pain dimensions.[84,85]

Psychological and behavioral mechanisms further modulate these physiological processes. Recent evidence indicates that sleep bruxism not only correlates with OFP and headache, but also with systemic health conditions and with COPCs, such as gastrointestinal, endocrine and sleep disorders.[18] Bruxism has been consistently linked to psychological stress and anxiety,[86,87] supporting its role as a behavioral manifestation of stress reactivity and autonomic dysregulation. These mechanisms are particularly relevant in women, in whom hormonal fluctuations heighten stress responsiveness and central sensitization, amplifying both muscular activity and pain perception.[88] The interplay of psychological stress, hormonal modulation and neuromuscular activation reinforces the need for integrated biopsychosocial approaches that combine behavioral, stress-reduction and somatic interventions.

Together, these biological and psychological mechanisms converge on common nociplastic pathways that integrate peripheral, hormonal and central sensitization processes. Table 4 summarizes the predominant mechanistic pathways linking key COPCs (IBS, GERD, PCOS, and endometriosis) with TMD. This overview integrates peripheral and central processes across hormonal, inflammatory and neuroplastic domains, illustrating how distinct conditions converge on shared nociplastic mechanisms.

Clinical implications

From a translational perspective, these mechanistic insights have direct clinical relevance. Evidence from Justribó-Manion et al. shows that subjects with higher baseline comorbidity severity and pain catastrophizing improved more when physical therapy was combined with behavioral interventions.[89] Moreover, prognostic modeling demonstrated that the number of coexisting COPCs is a robust clinical marker of OFP-related disability severity, with the predictive value comparable to psychosocial variables like pain catastrophizing or kinesiophobia.[89] This indicates that assessing comorbidity provides valuable information for identifying individuals at risk and guides appropriate patient management.

Table 4. Proposed mechanistic pathways linking chronic overlapping pain conditions (COPCs) with temporomandibular disorders (TMD)

COPC	Predominant mechanism	Proposed pathophysiological pathway	Link to TMD/OFP	Representative Evidence
IBS	Inflammatory/ Neuroimmune	Low-grade mucosal inflammation → cytokine release (IL-6, TNF-α) → central sensitization (trigeminal pathway)	Visceral–somatic cross-sensitization; chronic muscle pain	Aguilera-Lizarraga 2022[77]; Traub 2014[79]
GERD	Peripheral/ Inflammatory	Acidic reflux → mucosal irritation → afferent hyperexcitability and sleep disturbance	Reflux-related pH alteration may enhance orofacial sensitivity	Li 2025[81]; Manfredini 2023[80]
PCOS	Hormonal/ Inflammatory	Hyperandrogenism and insulin resistance → systemic inflammation → altered pain modulation	Estrogen/testosterone imbalance influencing joint and muscle pain	Yazici 2021[72]; Sun 2025[82]
Endometriosis	Hormonal/ Neuroimmune	Estrogen-dependent inflammation and pelvic–trigeminal cross-sensitization	Shared nociplastic mechanisms and cyclic pain exacerbation	Marciniak 2024[74]
IC / CPP	Neuroplastic/ Autonomic	Visceral hyperalgesia → increased afferent input to spinal and trigeminal nuclei	Central convergence leading to generalized hypersensitivity	Adams & Turk 2015[84]; Lam 2024[85]
Cognitive–Affective Modulation (all COPC)	Neuroplastic/ Psychosocial	Dysregulation of limbic and prefrontal circuits → altered pain modulation, catastrophizing, anxiety, and depression	Amplified pain perception and maintenance of chronicity through impaired descending inhibition	Lam 2024[85]; Adams & Turk 2015[84]; Justribó-Manion 2024[89]

This schematic synthesis facilitates a clearer understanding of how diverse systemic disorders contribute to orofacial pain through overlapping biological pathways. It also bridges the transition between basic neurobiological evidence and clinical management approaches.

Moreover, the effect sizes for the associations between TMD and both GERD and PCOS underscore the need for thorough differential diagnosis and multidisciplinary evaluation. Both GERD and PCOS can contribute to or mimic OFP symptoms, requiring coordinated care between dentistry, gastroenterology and gynecology. Treating the underlying condition, such as hormonal regulation in PCOS or reflux management in GERD, may reduce peripheral and central sensitization, improving OFP outcomes. Sun et al. found that treating PCOS in adolescents with TMJ anterior disc displacement improved condylar remodeling and reduced degenerative changes.[82] Similarly, systematic evidence indicates that proton pump inhibitors not only alleviate GERD symptoms, but may also improve TMD outcomes.[90]

These findings highlight the importance of considering COPCs in TMD assessment. Integrating multimodal management, including physical therapy, the medical treatment of systemic comorbidities, and psychosocial interventions, within interdisciplinary teams may improve symptom control and outcomes. Tailoring interventions to patient profiles, accounting for psychosocial distress and comorbidity severity, may be crucial for optimizing management and prognosis.

Clinically, integrating COPC assessment into TMD evaluation and management means:

– assessing COPCs: IBS, GERD, PCOS may contribute to OFP;
– tailoring interventions to patient profiles: consider psychosocial factors and comorbidity severity for prognosis;
– coordinating care between dentistry, gastroenterology, gynecology, and psychology/physical therapy; and
– targeting mechanisms: address peripheral (inflammation, hormonal dysregulation, reflux) and central (sensitization, maladaptive pain modulation) drivers.

Strengths and limitations

This review provides a quantitative synthesis of the COPC–OFP relationship, combining likelihood and prognostic perspectives, and applying GRADE to assess evidence certainty. Strengths include integrating longitudinal and cross-sectional designs to explore temporality and associations, rigorous selection criteria with the exclusion of duplicated cohorts, and sensitivity analyses to enhance robustness. Limitations must be acknowledged. The number of high-quality prospective studies was limited, restricting causal inference. Methodological heterogeneity in diagnostic criteria, outcome measurements and comparator characteristics reduced comparability, and may have introduced bias. Many studies lacked adequate control for confounders, which could inflate associations. Some analyses relied on few studies, limiting statistical power and precision. The available evidence is observational, so residual confounding cannot be fully excluded despite adjusted models and sensitivity analyses.

Our synthesis offers preliminary insights, but the associations should be interpreted with caution. These limitations were considered throughout the methodology and are reflected in the evidence certainty rating. Clinicians should view the findings as exploratory, while future research should prioritize prospective cohorts with standardized diagnostics, robust confounder adjustment and stratified analyses. Such improvements are needed to move beyond descriptive associations toward reliable evidence that can support precision management of TMD in patients with COPCs.

Conclusions

While this review provides the first quantitative synthesis of COPC–OFP associations, causal relationships remain to be confirmed through well-designed longitudinal studies. We found that IBS consistently increased the likelihood of both first-onset and chronic TMD, while GERD and PCOS also showed large effects, suggesting shared inflammatory, hormonal and pH-related pathways. These findings highlight the need for prospective, standardized studies and support a shift toward mechanism-based approaches in the prevention and treatment of chronic OFP.

Trial registration

The protocol of the study was registered with the International Prospective Register of Systematic Reviews – PROSPERO (registration No. CRD42024537553).

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

The authors used Paperpal for Word add-in (version 3.1; Cactus Communications Services, Mumbai, India) solely to improve the English language and the readability of the manuscript. Paperpal did not contribute to the scientific content, study design, data collection, data analysis, interpretation of the findings, or conclusions.

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Review

Classification of post-and-core assembly failures: An extensive compilation of 33 years of data

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Over the past 2 decades, a wide range of post-and-core materials has been developed to restore endodontically treated teeth (ETT) with extensive loss of coronal tooth structure. Although the prognosis and long-term success of ETT are influenced by multiple factors, most research has focused on post-and-core materials, the ferrule effect and luting agents. However, a classification of failures associated with post-and-core assemblies is currently lacking. This study aimed to identify and classify post-and-core assembly failures.

A literature search was conducted using the terms "post and core" and "dental post and core" in the title and abstract. Articles published in English between 1991 and 2024 were retrieved from the PubMed®, Scopus and Cochrane Library databases. Of the 491 articles identified, 23 met the inclusion criteria and were used to develop the proposed classification.

Based on the available evidence, post-and-core assembly failures can be classified into 2 categories: catastrophic and non-catastrophic failures. To the authors' knowledge, this is the first comprehensive classification of post-and-core assembly failures derived from evidence spanning 33 years of published literature. The proposed classification provides a standardized framework for identifying and reporting failures and may facilitate clinical decision-making, laboratory communication and future research.

Keywords: luting agents, ferrule, endodontically treated teeth, post-and-core failures, dental posts

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Highlights

- This article introduces the first comprehensive classification system for post-and-core assembly failures based on 33 years of published literature.
- The classification categorizes post-and-core assembly failures as catastrophic or non-catastrophic, providing a standardized framework for diagnosis, reporting and clinical decision-making.
- The classification supports consistent communication among clinicians, researchers and educators involved in post-and-core treatment.

Introduction

The restorative success of endodontically treated teeth (ETT) has been a subject of considerable debate over the past 2 decades. Because non-vital teeth are generally more susceptible to fracture than vital teeth,[1] a multifactorial approach is essential to ensure long-term survival of ETT. The prognosis of ETT depends on several factors,[2] and careful evaluation of each of these factors enables the clinician to assess the prognosis, determine the complexity of treatment[3] and develop an appropriate treatment plan.

Traditionally, it has been assumed that an endodontically treated tooth with extensive loss of coronal tooth structure requires the use of an anchorage from the root canal to improve strength and function of the restoration. However, Trope et al. demonstrated that preparation of the root canal for post placement weakens the tooth and reduces its resistance to functional forces.[4] It is also commonly believed that pulpless teeth become brittle and more prone to fractures due to reduced moisture content. However, only minor differences were noticed in the hardness and microhardness of dentin between vital and non-vital teeth.[5] Assif and Gorfil stated that the loss of dentin during access cavity preparation and post space preparation is the primary factor compromising the survival of ETT.[6] Hence, performing restorative procedures that preserve the remaining dentin thickness is more crucial than strengthening the tooth. [6]

The wide variety of available post materials and restorative techniques has resulted in conflicting recommendations regarding the optimal restorative approach. A systematic review evaluating the biomechanical considerations for restoring ETT concluded that interfacial defects and severe tooth breakdown associated with post failure occurred more frequently with rigid metal and ceramic posts.[5] However, fiber posts combined with composite resin demonstrated favorable clinical performance because their mechanical properties are more similar to those of dentin. It was also established, based on fatigue studies, that post placement is unnecessary when an adequate ferrule and sufficient coronal tooth structure are present. Nevertheless, when a post is required to improve the stability of the tooth, a resin post must be used along with an adhesive luting agent.[5]

Another systematic review evaluating the clinical performance and failure modes of pulpless teeth restored with posts found no significant differences in the survival rates of metal and fiber posts.[7] The authors concluded that both post systems demonstrate comparable clinical performance during short- to medium-term follow-up.[7]

Although several studies have supported the use of posts,[8–12] whereas others have questioned their placement,[13–17] there is currently no standardized classification of post-and-core failures. Such a classification would facilitate communication among clinicians and provide a standardized framework for research. A simple and reproducible classification describing both the type and severity of post-and-core failures could be useful for surveys, clinical trials, treatment planning, and outcome assessment.

Following a review of the published literature on post-and-core failures and after evaluation of the available evidence, the authors propose a new classification system for post-and-core failures together with recommendations for their management. To the best of our knowledge, no standardized classification of post-and-core failures has been described in the literature. The proposed classification categorizes failures into 2 broad groups, catastrophic and non-catastrophic, based primarily on whether the affected tooth can be salvaged. This classification is intended to assist clinicians in determining whether the tooth requires further treatment or needs to be extracted. Catastrophic failures comprise those resulting in irreparable damage to the tooth, whereas non-catastrophic failures include conditions in which the tooth can be salvaged even after the failure.

Material and methods

Search methodology

A literature search was conducted using the following search terms: ("post and core"(Title/Abstract)) OR ("dental post and core"(Title/Abstract)). Articles published in English between 1991 and 2024 were retrieved from the PubMed®, Scopus and Cochrane Library databases. The identified studies were screened according to the predefined inclusion and exclusion criteria.

Inclusion and exclusion criteria

The inclusion criteria encompassed randomized controlled trials (RCTs), quasi-RCTs, in vivo or in vitro studies, retrospective or prospective studies, cohort studies, case–control studies, systematic reviews, or meta-analyses that evaluated and described the types of post-and-core failures and their management. Literature reviews, scoping reviews, narrative reviews, case reports, and case series were excluded.

Data collection

A total of 511 records were identified across all databases on April 10, 2024. After removal of duplicate records, 491 articles remained for title and abstract screening. Following screening and application of the inclusion and exclusion criteria, 23 studies were included in the final analysis (Fig. 1). These 23 articles were used to formulate the proposed classification system (Table 1).[11,12,18–38]

Results

Classification of post-and-core failures

Post-and-core failures can be broadly classified into 2 major categories: catastrophic and non-catastrophic failures (Fig. 2,3). Each category is further subdivided according to the type and extent of failure. The proposed classification was developed from evidence reported in 23 studies conducted in both clinical and laboratory settings. Hence, it encompasses the various types of failure that may occur under laboratory conditions, in clinical setting, or both. A comprehensive classification of post-and-core failures has the potential to standardize the reporting of outcomes in future RCTs, in vitro studies, cohort and case–control studies, systematic reviews, and meta-analyses. In addition, in may facilitate scientific communication among clinicians, researchers and dental technicians (Table 2,3).

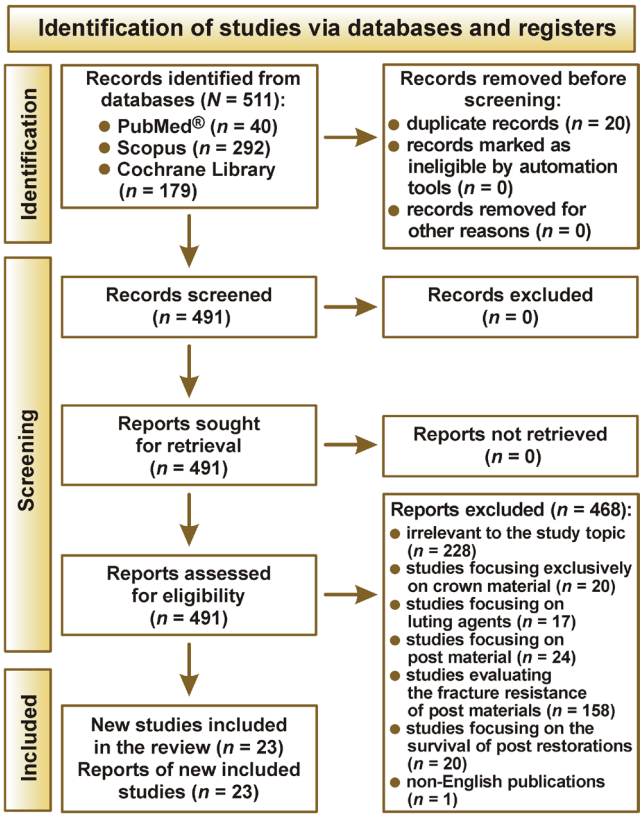


Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram illustrating the study selection process



Fig. 2. Catastrophic post-and-core assembly failures and their subdivisions

Class IA – vertical root fracture; Class IB – horizontal root fracture; Class IC – oblique root fracture; Class ID – crown/fixed partial denture (FPD)/core fracture; Class IE – root perforation during post placement.

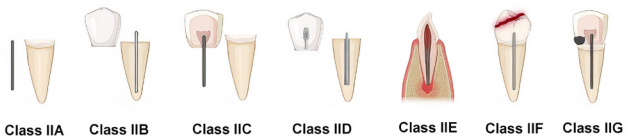


Fig. 3. Non-catastrophic post-and-core assembly failures and their subdivisions

Class IIA – post dislodgement; Class IIB – crown dislodgement with or without core debonding; Class IIC – post-and-core assembly dislodgement; Class IID – crown/FPD/core fracture; Class IIE – endodontic failure; Class IIF – crown chipping; Class IIG – secondary caries.

Discussion

The proposed classification identifies the spectrum of failures that may occur in ETT restored with a post-and-core system. It may serve as a practical framework for evaluating outomes in a laboratory setting, predicting prognosis, establishing a diagnosis, formulating treatment plans, and facilitating communication among clinicians. The classification was developed following a critical appraisal of evidence from prospective and retrospective studies, RCTs, in vitro investigations, systematic reviews, and meta-analyses.

There remains no clear consensus regarding the role of post-and-core restorations in improving the clinical outcomes of ETT. Numerous studies have focused on identifying the post-and-core material that provides the greatest fracture resistance and lowest failure rate. However, the prognosis of ETT is influenced by several additional factors, including the periapical status, tooth position, occlusal contacts, collagen degradation, and intermolecular cross-linking of the tooth root dentin.[39]

Table 1. Studies evaluating different types of post-and-core failures

Study	Objectives	Type of failure	Research methodology	Conclusions
Sorensen and Martinoff 1984[18]	To determine the clinical significance of post reinforcement and coronal coverage in ETT.	• post dislodgement • root fractures • root perforation	retrospective study	Intracoronal reinforcement did not significantly increase resistance to fracture or dislodgement. Coronal coverage did not significantly improve the clinical success of anterior teeth but significantly improved the success rate of posterior teeth.
Assif et al. 1993[19]	To evaluate the effect of post design on the fracture resistance of endodontically treated premolars restored with cast crowns.	• oblique root fracture	prospective study	Post design did not influence the fracture resistance of ETT restored with complete cast crowns.
Torbjörner et al. 1995[20]	To investigate the failure rate and failure characteristics of 2 post designs.	• loss of retention • root fracture	retrospective study	ParaPost restorations with cast cores demonstrated a higher success rate than tapered cast post-and-core restorations.
Akkayan and Caniklioglu 1998[21]	To evaluate the effect of 4 different post systems on the fracture resistance of crowned ETT.	• vertical root fracture • horizontal root fracture	prospective study	The choice of prefabricated post systems is important in determining the maximum resistance to fracture in ETT.
Ferrari et al. 2000[22]	To assess the treatment outcomes of cast post-and-core and Composipost systems.	• root fracture • crown dislodgement • endodontic failure	retrospective study	Composipost systems demonstrated superior clinical performance compared with conventional cast post-and-core restorations.
Heydecke et al. 2001[23]	To compare the fracture strength and survival rate of crowned maxillary incisors with approximal Class III cavities using different core build-up techniques.	• oblique root fracture • bent posts • post dislodgement from the root canal • post fracture	prospective study	Enlargement of the root canal after endodontic treatment should be avoided, as it cannot be compensated for by the use of composite resin. Specimens restored without posts exhibited fewer catastrophic failures.
Hu et al. 2003[24]	To evaluate the fracture resistance and failure modes of ETT restored with 4 post-and-core systems.	• catastrophic failures (predominantly root fractures) observed in all groups • higher incidence of catastrophic root fractures in groups restored with ceramic posts and resin composite cores	prospective in vitro study	The fracture resistance of all 4 post systems was considered clinically acceptable. Unfavourable tooth fractures were observed in all groups.
Fokkinga et al. 2006[25]	To investigate the in vitro fracture behavior of severely damaged maxillary premolars restored with different post-and-core systems and covered with full metal crowns.	• post and crown displacement • root fracture • root fractures with or without post and crown displacement	prospective study	Fracture resistance and failure mode of severely damaged premolars restored with adhesively cemented crowns and a limited ferrule were independent of the post-and-core system used.
Salvi et al. 2007[12]	To assess the survival rates and complications of root-filled teeth restored with or without post-and-core systems over a 4-year period.	• root fracture • secondary caries • post-treatment radicular disease • loss of retention	prospective study	Reported survival rates were 92.5% for titanium posts, 94.3% for teeth restored without posts, and 97.1% for cast post-and-core restorations.
Peutzfeldt et al. 2008[26]	To identify the factors associated with failure and characterize the failure modes of post-retained restorations.	• tooth fracture • post loosening • post fracture	prospective study	Tooth fracture was the most common failure, followed by post loosening and post fracture.
Al-Wahadni et al. 2008[27]	To investigate the fracture resistance and failure modes of teeth restored with different prefabricated post systems.	• combined root and core fractures (70%) • root fractures (16%) • core fractures (13%)	prospective study	Teeth restored with titanium posts demonstrated higher fracture resistance than those restored with carbon fiber or glass fiber posts. Most failures were catastrophic, rendering the teeth non-restorable.
Gómez-Polo et al. 2010[28]	To compare the cumulative survival rates of teeth restored with prefabricated posts and Co–Cr cast post-and-core restorations.	• post dislodgement • root fracture • caries	retrospective study	Cast post-and-core restorations appeared to be less retentive than prefabricated posts. Root fractures and caries were common causes of treatment failure in teeth restored with prefabricated posts.
Schmitter et al. 2011[29]	To compare the survival rates of 2 different post systems after 5 years of service.	• post-and-core assembly loosening • loss of retention of fiber posts • chipping or fracture of single crowns • root fracture • loss of retention of the post and crown	prospective study	Teeth with extensive coronal structure loss and those restored with metal screw posts exhibited a higher risk of failure.
Zicari et al. 2011[30]	To compare the 3-year clinical outcomes of glass fiber posts and composite cores with gold alloy-based post-and-core restorations in ETT.	• absolute failures: 2 root fractures and 1 endodontic failure • relative failures: 3 cases of post/core retention loss and 1 post fracture	prospective clinical trial	Both cast gold and composite post-and-core systems demonstrated satisfactory clinical performance. The overall success and survival probabilities at 3 years were 91.7% and 97.2%, respectively.

Study	Objectives	Type of failure	Research methodology	Conclusions
Balkenhol et al. 2011[31]	To evaluate the influence of 2 post materials, preparation design and luting agent on the survival probability of root-filled teeth restored with all-ceramic restorations.	• CeraPost: debonding was the most common failure • DentinPost: post fracture was the most common failure	prospective in vitro study	Teeth restored with a ferrule preparation design exhibited higher survival rates. The luting agent had only a minor importance on survival, whereas post material and ferrule design were the most influential factors. DentinPosts demonstrated superior results.
Ferrari et al. 2012[11]	To evaluate the effect of the amount of remaining coronal dentin and the placement of prefabricated and/or customized fiber posts in endodontically treated premolars after 6 years.	• in the group without posts, the highest incidence of root fractures and crown dislodgement was observed • in the group restored with prefabricated posts, post decementation was mainly observed • teeth with 1, 2 and 3 remaining coronal walls exhibited significantly lower failure rates	prospective study	Preservation of at least 1 coronal wall significantly reduced the risk of failure, regardless of the restorative procedure.
Sterzenbach et al. 2012[32]	To compare the survival rates of abutment teeth with severe coronal tissue loss and 2 or fewer remaining cavity walls restored with either prefabricated glass fiber-reinforced epoxy resin posts or titanium posts luted with a self-adhesive material.	• glass fiber-reinforced epoxy resin posts: 3 root fractures and 1 core fracture • titanium posts: 3 endodontic failures	prospective study	Regardless of post material or rigidity, post-endodontic restorations achieved high long-term survival in severely damaged abutment teeth with two or fewer remaining coronal walls and a 2-mm ferrule.
Hegde et al. 2012[33]	To evaluate the fracture strength and failure modes of ETT with flared root canals restored with glass fiber, quartz fiber and cast base-metal post-and-core systems.	• cast metal posts showed 86.66% non-repairable failures and 13.34% repairable failures • both glass fiber and quartz fiber posts demonstrated 100% repairable fractures	prospective in vitro study	Teeth restored with cast posts demonstrated approximately twice the fracture strength of those restored with fibre posts. Failures associated with fibre posts were repairable, whereas failures associated with cast posts were predominantly non-repairable.
Gbadebo et al. 2014[34]	To compare the clinical performance of metallic and glass fiber posts used for the restoration of ETT.	• in the metallic post group, 1 tooth failed because of decementation of the PFM crown	prospective study	The survival rate was 100% for the fiber-reinforced post group and 97.5% for the ParaPost group; however, the difference was not statistically significant.
Sarkis-Onofre et al. 2014[35]	To compare the survival of glass fiber and cast metal posts used to restore ETT with no remaining coronal walls.	• 2 glass fiber posts debonded • 1 glass fiber post debonded in association with a root fracture • 1 cast metal post was associated with a root fracture	prospective study	The 3-year recall rate was 92.3%, and the survival rates of glass fiber and metal posts were comparable. Post type did not significantly influence restoration survival.
Cloet et al. 2017[36]	To compare the 5-year outcomes of glass fiber composite and cast post-and-core restorations in ETT.	• root fracture • post fracture within the root canal • caries • endodontic failure • periodontal failure • loss of retention of the post	prospective study	After a 5-year follow-up, cast gold and composite post-and-core restorations supporting ceramic crowns with an adequate ferrule demonstrated comparable clinical performance, with success and survival rates of 85.2% and 91.5%, respectively.
Casanova and Özcan 2021[37]	To investigate the load-bearing capacity of intraradicular posts and core materials under static and dynamic loading conditions.	• the titanium post group presented with cohesive failure within the crown material with adhesive detachment from the post and core • remaining post groups showed irreparable failures of the crown and core from the cervical coronal dentin surface	prospective in vitro study	Placement of woven fiber within the core and coronal region did not reinforce the restoration and predominantly resulted in adhesive failure of the crown and core. In the absence of coronal tooth structure, intraradicular posts provided a stable foundation for the core and crown assembly.
Nodehi et al. 2022[38]	To compare the effect of different post-and-core systems (Co–Cr, NPG casting, CAD–CAM) on the fracture toughness and failure modes of root canal-treated premolars.	• catastrophic fractures were observed in 80% of samples restored with Co–Cr and NPG castings • 1 horizontal and 1 vertical root fracture were observed in the CAD–CAM group	prospective in vitro study	CAD–CAM-milled restorations demonstrated the highest fracture strength and the lowest risk of catastrophic failure. CAD–CAM restorations exhibited the highest frequency of restorable failure modes.

Co–Cr – cobalt–chromium; PFM – porcelain-fused metal; NPG – non-precious gold; CAD–CAM – computer-aided design/computer-aided manufacturing.

Table 2. Classification of catastrophic post-and-core assembly failures and their subdivisions

Subdivision	Type of failure	Explanation
Class IA	vertical root fracture	The post fractures together with the root, resulting in a vertical root fracture. This fracture may occur either at the level of the post (below the CEJ) or at the level of the core, crown or FPD, extending into the root. This type of failure may occur in both clinical and laboratory settings.
Class IB	horizontal root fracture	The post fractures together with the root, resulting in a horizontal root fracture. This type of failure may occur in both clinical and laboratory settings.
Class IC	oblique root fracture	The post fractures together with the root, resulting in an oblique root fracture. This fracture may occur either at the level of the post (below the CEJ) or at the level of the core, crown or FPD, extending into the root. This type of failure may occur in both clinical and laboratory settings.
Class ID	crown/FPD/core fracture	This subdivision applies only to bonded posts, for which post retrieval is difficult. The post fractures at or above the level of the CEJ, involving the crown, core or FPD. This type of failure may occur in both clinical and laboratory settings.
Class IE	root perforation during post placement	This is an iatrogenic failure that may occur in both clinical and laboratory settings.

CEJ – cementoenamel junction; FPD – fixed partial denture.

Table 3. Classification of non-catastrophic post-and-core assembly failures and their subdivisions

Subdivision	Type of failure	Explanation	Treatment
Class IIA	post dislodgement	The entire post either debonds or becomes dislodged without fracturing the crown or the root. This type of failure may occur only in a laboratory setting.	none
Class IIB	crown dislodgement with or without core debonding	Only the crown becomes dislodged, either with or without debonding of the core from the post. The post remains intact within the root canal without catastrophic damage to the tooth. This type of failure may occur in both clinical and laboratory settings.	If only the crown is dislodged, recement the crown after evaluating and correcting any occlusal interferences. If both the crown and core are dislodged, identify the underlying cause, remove the existing core material from the internal surface of the crown, rebuild the core, and recement the crown.
Class IIC	post-and-core assembly dislodgement	The entire post-and-core assembly debonds and is displaced as a single unit. This type of failure may occur in both clinical and laboratory settings.	Identify the cause of failure and recement the post-and-core assembly.
Class IID	crown/FPD/ core fracture	In this type of failure, the crown, core or FPD fractures together with part of the post, while the remaining portion of the post is still present in the root canal. This subdivision applies only to non-bonded posts, such as serrated or screw-type posts, for which post retrieval is still possible without causing catastrophic damage to the remaining tooth structure. This type of failure may occur in both clinical and laboratory settings.	Retrieve the remaining post from the root canal while preserving as much radicular dentin as possible, and repeat the post-and-core procedure.
Class IIE	endodontic failure	This type of failure includes apical periodontitis or an endodontic-periodontal lesion affecting a tooth restored with a post-and-core assembly. It occurs only in the clinical setting.	Apical periodontitis: Depending on the type of post used, treatment may consist of either orthograde endodontic retreatment or retrograde apicoectomy. If post removal is likely to cause excessive damage to the remaining tooth structure, retrograde apicoectomy is the preferred treatment. Endodontic-periodontal lesion: Treatment depends primarily on the periodontal status of the tooth. If the lesion is associated with significant mobility, extraction may be the only treatment option.
Class IIF	crown chipping	This type of failure involves fracture of the porcelain without causing catastrophic damage to the tooth or the post-and-core assembly. It may occur in both clinical and laboratory settings.	Repair of the fractured or chipped porcelain may be performed using a porcelain repair kit.
Class IIG	secondary caries	This type of failure involves the development of secondary caries in a tooth restored with a post-and-core assembly. It occurs only in the clinical setting.	Treatment depends on the extent of the carious lesion and may range from replacement of the restoration to extraction of the tooth.

The primary purpose of a post is to retain the core in teeth with extensive loss of coronal tooth structure rather than to reinforce the remaining tooth structure.[6] Post placement has been shown to reduce the incidence of catastrophic failures in selected ETT. However, when 3 or 4 coronal walls remain intact, neither post placement nor the type of post-and-core material appears to significantly influence the clinical outcome.[39]

A nationwide survey conducted among German dentists in 2006 reported that 52% of respondents routinely placed a post in every second endodontically treated tooth, while almost 54% believed that posts reinforce ETTs.[2] Current

evidence, however, indicates that unnecessary post placement increases the risk of root perforation and may predispose the tooth to root fracture.[1,40–42]

Although in vitro studies provide valuable information regarding the prognosis and prediction of clinical outcomes of ETT, RCTs produce the highest level of clinical evidence. A 17-year follow-up of a RCT by Fokkinga et al. on ETT with and without prefabricated metal posts concluded that placement of a post did not significantly influence tooth survival.[43] Similar findings were reported by Mannocci et al.[44] However, a RCT by Zicari et al. noted a 3-year survival probability of 97.2% and a success rate of 91.7% for restorations supported by glass fiber and gold alloy-based posts, respectively.[15] Similar results were reported in another clinical trial,[45] in which post-endodontic restorations exhibited a failure rate of only 6.42% after 3 years.

Bitter et al. in their in vitro study reported that self-adhesive resin cements produced only a modest increase in the retentive strength of the dowel.[17] Conversely, Mazzoni et al. demonstrated significantly reduced retention and increased interfacial nanoleakage with 2 self-adhesive resin cement.[46] Balkenhol et al. investigated the influence of luting agents under cyclic loading conditions and concluded that the choice of luting agent had a minor influence on the survival of post-and-core restorations.[31]

As far as luting agents are concerned, the survival of the post-and-core assembly depends largely on the accuracy of each clinical step and the operator's handling of the procedure. In addition to technical challenges involved, the limited access within the root canal space further complicates the bonding procedure. To better understand the effect of luting agents on the survival of the post-and-core assembly, long-term clinical investigations in the form of RCTs are required; however, such evidence is currently lacking.

Although several RCTs have investigated the performance of post-and-core restorations, definite conclusions regarding the optimal restorative strategy remain elusive. The success or failure of these restorations depends on numerous factors. Therefore, additional well-designed, comprehensive RCTs that account for these variables are required to reach a consensus. At present, the available literature remains heterogeneous, contributing to continued uncertainty regarding the indications for post placement, the selection of rigid versus flexible posts, the importance of the ferrule effect, and the optimal luting agent.

Conclusions

Until comprehensive RCTs evaluating all relevant factors affecting the success and failure of post-and-core restorations become available, consensus regarding the indications for post placement is unlikely to be achieved. Future in vitro studies and well-designed clinical trials are needed to validate the proposed classification and assess its reliability and clinical applicability. Based on the currently available evidence, the authors propose a novel classification of post-and-core failures that may provide a standardized approach for classifying the type of post-and-core failures, predicting prognosis, and guiding treatment planning.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

Not applicable.

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Review

Use of erbium lasers to prepare cavities in deciduous teeth for caries treatment: A systematic review

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Abstract

Control of the physical parameters of Er:YAG and Er,Cr:YSGG laser settings may optimize and improve their effectiveness for cavity preparation in deciduous teeth while ensuring patient comfort and safety during treatment.

This systematic review aimed to identify the optimal ranges of laser parameters (frequency, power, applicator type and diameter, pulse duration, and water spray settings) for Er:YAG and Er,Cr:YSGG lasers used in the treatment of carious lesions involving the enamel and dentin of deciduous teeth in children.

Scientific databases were systematically searched, and the included articles were assessed for risk of bias. The Cochrane risk-of-bias tool was modified according to the requirements of this systematic review.

Ten randomized studies met the inclusion criteria. Four were in vitro studies conducted on extracted deciduous teeth, and 6 were clinical trials involving children. Er:YAG lasers were used in 6 studies, whereas Er,Cr:YSGG lasers were used in 4 publications.

The available evidence suggests that Er,Cr:YSGG laser-assisted cavity preparation in deciduous teeth may be more effective than conventional rotary instrumentation in reducing anxiety and pain in young patients. However, based on the analyzed studies, it is not possible to establish optimal laser parameters for the preparation of carious enamel and dentin in deciduous teeth using either Er:YAG or Er,Cr:YSGG lasers. Furthermore, the included studies provide limited information regarding the laser parameters used.

Keywords: pain, dental caries, primary teeth, lasers, efficacy

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Highlights

- This systematic review identified only 10 randomized studies evaluating Er:YAG and Er,Cr:YSGG lasers for cavity preparation in primary teeth, highlighting the limited available evidence.
- Er,Cr:YSGG laser-assisted cavity preparation reduces pain and anxiety and improves patient acceptance compared with conventional rotary instrumentation, despite longer treatment times.
- Heterogeneous study designs and incomplete reporting prevent identification of evidence-based laser settings for cavity preparation in primary enamel and dentin.
- Future randomized clinical trials should standardize reporting of laser parameters (power, energy, pulse duration, applicator characteristics, and water/air settings) to establish clinically applicable protocols for cavity preparation in primary teeth.

Introduction

The treatment of carious disease in deciduous teeth consists of the limited removal of diseased, cariously altered tooth tissues and replacing them with a filling material. It is aimed at inhibiting the caries process, restoring the tooth function, preserving the vital pulp, and preventing the formation of secondary caries. Therefore, the currently used methods of managing dental caries follow the principles of minimally invasive dentistry (MID).[1–3]

The methods of minimally invasive treatment of carious cavities include pit and fissure sealing, caries infiltration, preventive resin restoration (PRR-1 and PRR-2), atraumatic restorative treatment (ART), chemomechanical caries removal (CMCR), air abrasion, kinetic cavity preparation (KCP), laser cavity preparation, sonic cavity preparation, ultrasonic cavity preparation, and steel crowns.

One method of treating carious cavities in deciduous teeth is the use of erbium lasers (Er:YAG and Er,Cr:YSGG) for the preparation of hard dental tissues. This is possible due to the presence of water in hard tissues, which is the main chromophore that efficiently absorbs the energy of the electromagnetic wave generated by these lasers (2,940 nm for Er:YAG, close to the maximum absorption of water; 2,780 nm for Er,Cr:YSGG). It is also worth mentioning the second chromophore, hydroxyapatite, which also absorbs these wavelengths well and is abundant in the hard tissues of teeth. The Er:YAG and Er,Cr:YSGG lasers are the most used and safest lasers for the preparation of dental hard tissues. They are characterized by the highest absorption in water and hydroxyapatite compared with the Nd:YAG laser (1,064 nm) and the CO_2 laser (10,600 nm).[4–7]

The first mechanism of action of the high-power laser occurs through the obliteration of tubules by the partial fusion of dentin substrates. The low-power laser decreases the chronic inflammatory process present in the cells by activating sodium and potassium pumps and obliterating dentinal tubules through the formation of tertiary dentin. It can also promote analgesic, anti-inflammatory and biomodulatory effects.[8] The second mechanism of action of the high-power laser is based on the ablation phenomenon. The tissue is exposed to laser radiation, causing the water contained within it to be rapidly transformed into steam, and the tissue is fragmented into fine particles. Ablation makes the procedure very precise. If the laser pulses are of high power (high energy and short duration), the rate of ablation is greater than the rate at which heat penetrates the tissues. The penetration depth of the Er:YAG laser in enamel is 7 μm, which is 3 times smaller than that of the Er,Cr:YSGG laser. In dentin, the penetration depth of the Er:YAG laser is 5 μm and is also 3 times shallower than that of the Er,Cr:YSGG laser. The ablation threshold is the minimum amount of laser radiation pulse energy required to initiate the ablation process. The ablation threshold for enamel when using Er:YAG and Er,Cr:YSGG lasers is 12–20 J/cm^2, and for dentin, 8–14 J/cm^2.[9] The most important factor is to deliver adequate energy in the shortest possible time to achieve effective ablation without damaging the tissues. Laser treatment is well tolerated by patients and is considered safe, quick and painless.[8]

The main chromophore in enamel and dentin for both the Er:YAG and Er,Cr:YSGG lasers is the water present between the hydroxyapatite crystals. Hydroxyapatite itself is also a chromophore. It is very important to consider the differences in water content between enamel and dentin. It is also necessary to compare the structural differences between primary and permanent teeth, as well as between immature or newly erupted teeth and aged teeth.

Healthy enamel is a highly mineralized tissue composed of 85% hydroxyapatite, 12% water and 3% organic tissue. Healthy dentin is less mineralized than enamel. It contains approx. 45–47% mineral components, 30–33% organic tissue and 20–24% water. The water content of carious tissue ranges from approx. 27% to 54%, which is higher than that of healthy tissues and depends on the stage of the carious lesion. Differences in the content of the main chromophore within the tissue determine the selection of the appropriate laser parameters according to the tissue being prepared. As a rule, the more hydrated the tissue, the lower the laser parameters should be. This is the main reason why the laser energy level must be decreased during dentin ablation, because both healthy and carious dentin have a higher water content than enamel.

Primary teeth have a higher water content in enamel and dentin. Additionally, the enamel layer is much thinner than that of permanent teeth, which is why ablation occurs much more rapidly than in the enamel of permanent teeth.

The use of the Er:YAG laser or the Er,Cr:YSGG laser to prepare carious cavities in primary teeth must be performed using appropriate parameters. The energy level and frequency should be lower than those used for the treatment of permanent teeth. Other settings are also very important, including the water spray, air cooling, and the type and diameter of the laser applicator.

Primary teeth have several ultrastructural differences compared with permanent teeth (Table 1).[10,11]

Advantages of the use of erbium lasers include:

- the laser beam can operate in contact or non-contact mode;
- the laser tip does not have to touch the tooth; cutting efficiency is greatest when the tip is positioned approx. 1 mm from the target tissue;
- the ideal impact angle for laser ablation of enamel is perpendicular to the orientation of the enamel prisms;
- reduction of bacteria: a study by Hibst et al.[12] showed that bacteria beneath the prepared surface were eliminated during laser cavity preparation to a depth of 300–400 µm;
- reduction of bacteria in combination with the use of antiseptics and lasers was reported by Golob Deep et al.[13] They also demonstrated a reduction in dentin hypersensitivity using high-power lasers. For this purpose, high-power lasers were applied for an average of 43 s, delivering 1–20 pulses during a single visit, as reported by Pion et al.[8];
- minimal heat increase in the pulp chamber;
- no smear layer after cavity preparation with laser use;
- no vibration.

Due to the differences in wave absorption, although the described lasers belong to the same family, there are important differences between them. Table 2 presents a comparison of the properties of the Er:YAG and Er,Cr:YSGG lasers.[9,14–17]

Our study aims to critically evaluate, through a systematic review of the literature, randomized clinical trials with respect to the setting parameters of erbium lasers (Er:YAG and Er,Cr:YSGG) in the conservative treatment of deciduous teeth in children.

Material and methods

Focused question

Is it possible to precisely determine the optimal ranges of Er:YAG and Er,Cr:YSGG laser settings (energy, power, frequency, applicator size and diameter, and amount of air-water spray) for the preparation of cariously affected enamel and dentin in deciduous teeth?

Protocol, information sources, search strategy, and study selection

The review was prepared in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines.[18] Details of the study protocol are presented in Table 3.

Electronic searches were conducted from 2001 to May 29, 2024. The PubMed® and Cochrane Library databases were searched using the following Medical Subject Headings (MeSH) terms, keywords, and their combinations: ("Erbium YAG laser" OR "Er:YAG laser" OR "Er-YAG

Table 1. Structure of primary teeth[10,11]

Layer	Characteristics
Enamel	• less mineralized than permanent teeth
	• has a thinner enamel layer than permanent teeth
	• has a higher water content than permanent teeth
	• following laser irradiation, the enamel surface appears disorganized and irregular, lacking the typical pattern observed in permanent teeth
	• the surface often contains a superficial aprismatic layer, making primary teeth appear whiter and more opaque than permanent teeth
	• the prism diameter is 2.9 ±1.2 µm
	• the angulations of the prisms vary along the crown of the tooth and are more perpendicular in the cervical area than in the coronal area (approx. 70°)
	• enamel mineralization in primary second molars is completed by the end of the first year of life
Dentin	• has a higher water content than primary enamel
	• contains dentinal tubules of different sizes
	• dentinal tubules are smaller in diameter and more widely spaced than those in permanent teeth
	• contains fewer dentinal tubules per unit area than permanent teeth
	• dentinal tubules follow a straighter course, which may contribute to the more rapid progression of caries in primary teeth
	• no significant differences in the occurrence of interglobular dentin; however, the interglobular dentin is oriented at an angle in primary teeth
	• following laser irradiation, the primary dentin surface appears irregular with open dentinal tubules
	• intertubular dentin contains more water and undergoes greater ablation than peritubular dentin

Table 2. Comparison of the properties of Er:YAG and Er,Cr:YSGG lasers[8,15,16]

Variable		Er:YAG laser (2,940 nm)	Er,Cr:YSGG laser (2,780 nm)
Laser body		YAG crystal (yttrium–aluminum–garnet) with the admixture of Er^{3+} ions	YSGG crystal with the admixture of Er^{3+} ions
Mode of operation		pulse	pulse
Pulse duration (pulse width)		can operate at pulse durations below 100 µs	limited to pulse durations above 400 µs
Effectiveness in tissues	enamel	60% more effective	60% less effective
	dentin	30% more effective	30% less effective
Absorption coefficient	overall	3-fold higher than Er,Cr:YSGG	3-fold lower than Er:YAG
	enamel	150 mm^{-1}	50 mm^{-1}
	dentin	200 mm^{-1}	66.5 mm^{-1}
Wave absorption		considerably higher in enamel and dentin than Er,Cr:YSGG	significantly lower in enamel and dentin than Er:YAG
Degree of ablation	enamel	5-fold higher (very high)	5-fold lower
	dentin	3.7-fold higher (tall)	3.7-fold lower
Heat deposition in hard dental tissues		low or absent	higher
Penetration depth	enamel	7 µm (3-fold shallower penetration)	21 µm (3-fold deeper penetration)
	dentin	5 µm (3-fold shallower penetration)	15 µm (3-fold deeper penetration)
QSP mode		yes	no
Smear layer after cavity preparation		absent	absent

If high-power laser pulses (high energy and short duration) are used, the rate of ablation exceeds the rate of heat diffusion into the surrounding tissues.
QSP – quantum square pulse.

Table 3. Characteristics of the Er:YAG laser (wavelength: 2,940 nm) settings used for different tooth surfaces

Surface		Frequency [Hz]	Pulse of energy [mJ]	Type of applicator	Liquid water [mL/min]	Air cooling [L/min]	Pulse duration [µs]
Enamel	occlusal surface	10	160	C400F	2.5–3.0	7	300
			230	C600F			
	proximal surface	10	160	C400F	2.5–3.0	7	300
			230	C600F			
	labial or palatal surface of anterior teeth	10	150	C600F	2.5–3.0	7	300
			180	C800F			
Dentin	occlusal surface	10	120	C600F	2.0–2.5	7	300
			150	C800F			
	proximal surface	10	120	C600F	2.0–2.5	7	300
			150	C800F			
	labial or palatal surface of anterior teeth	10	120	C600F	2.0–2.5	7	300
			150	C800F			
Surface modification	every surface	20	50	C400F	2.5–3.0	7	300
			70	C600F			

laser" OR "Er,Cr:YSGG" OR "Erbium Chromium YSGG" OR "2940 nm" OR "2780 nm") AND ("primary teeth" OR "deciduous teeth" OR "caries" OR "enamel" OR "dentin").

After applying the exclusion criteria (publications from the last 23 years (2001–2024), English language, and randomized trials only), the initial 1,501 articles retrieved from PubMed® were reduced to 138, and the initial 232 articles retrieved from the Cochrane Library were reduced to 28. During the initial screening phase, the titles and abstracts of these articles were independently screened and assessed by 2 reviewers according to the same criteria. Any disagreements were satisfactorily resolved through discussion.

A duplicate filter was then applied, resulting in the removal of 5 publications and leaving 161 articles for further evaluation.

After narrowing the selection criteria, 140 articles on permanent teeth and adhesion were excluded.

Subsequently, the full texts of the 21 articles were examined, and a final exclusion of 11 studies was made. Ultimately, 10 publications were included in the review (Fig. 1).

Eligibility criteria

The following inclusion criteria were considered in the review:

- randomized trials only;
- use of the Er:YAG or Er,Cr:YSGG laser for the preparation of carious cavities in the deciduous teeth of children;
- presence of a negative control group;
- at least 10 patients (10 teeth) included in the study group;
- reporting of the laser operating parameters (pulse duration, frequency, power, type of laser applicator, and amount of air-water spray);
- a minimum follow-up period of 3 months;
- in vivo and in vitro studies.

Studies were excluded if they used laser other than Er:YAG and Er,Cr:YSGG, were duplicate publications, reported data from studies with the same clinical approval number, or investigated the use of the Er:YAG or Er,Cr:YSGG laser in general medicine, cosmetology, aesthetic medicine, or the conservative treatment of permanent teeth.

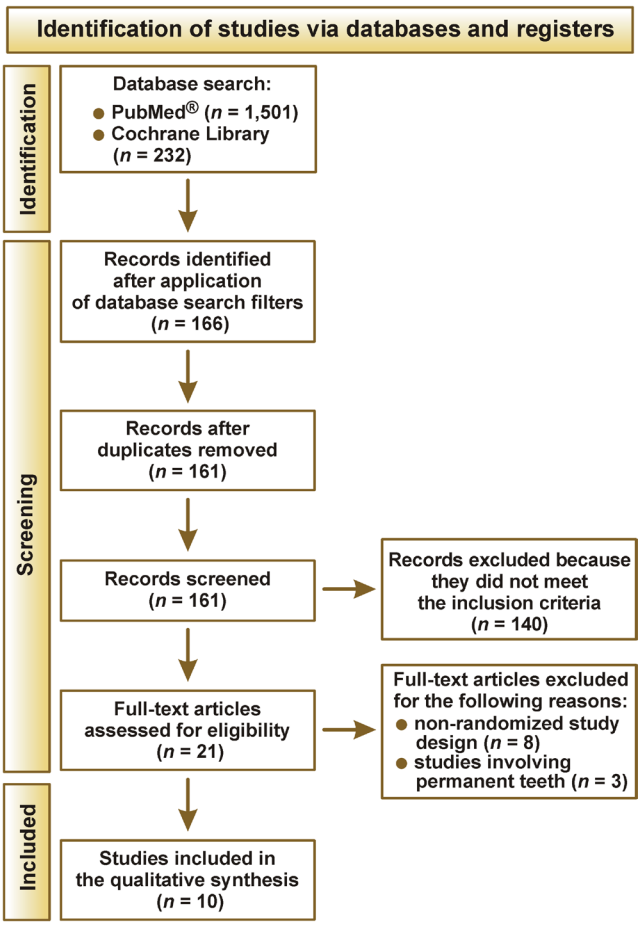


Fig. 1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flowchart illustrating the study selection process

Quality assessment and risk of bias across studies

Following data extraction, the included articles were further evaluated by assessing their risk of bias. The Cochrane risk-of-bias tool[17] was modified according to the requirements of this systematic review (Table 4). The studies were classified according to the total number of "YES = 1" responses to the posed questions.[19]

Table 4. Assessment of the risk of bias of the studies included in the review

Question	Valério et al.[20]	Rossi et al.[23]	Liu et al.[21]	Johar et al.[25]	Wanderley et al.[24]	Sungurtekin et al.[14]	Alia et al.[36]	Abdrabuh et al.[22]	Felemban et al.[26]	Wang et al.[27]
Was the trial randomized?	1	1	1	1	1	1	1	1	1	1
Was the test performed using a split-mouth design?	1	0	1	0	0	0	1	1	1	0
Were all laser parameters adequately described (pulse duration, frequency, power, type of laser applicator, and amount of air-water spray)?	1	1	1	1	1	1	0	1	0	1
Did the study group include more than 10 cases?	1	1	1	1	1	1	1	1	1	1
Was there a control group?	1	1	1	1	1	1	1	1	1	1
Were the study results statistically analyzed?	1	1	1	1	1	1	0	1	1	1
Were the baseline parameters compared with the outcome parameters?	1	0	0	0	0	0	0	0	0	0
Was the effect of dentin preparation on the pulp assessed?	0	0	0	0	0	0	0	0	0	0
Was a power meter used?	0	0	0	0	0	0	0	0	0	0
Total	7	5	6	5	5	5	4	6	5	5

For the current study, the risk of bias was assessed according to the following score ranges:
- high risk: 0–3;
- moderate risk: 4–6;
- low risk: 7–9.

After analyzing the quality of the publications, 1 study received a score of 7, corresponding to a low risk of bias.[20] Two studies received a score of 6, corresponding to a moderate risk of bias,[21,22] and the remaining 6 publications received 5 points, which also corresponded to a moderate risk of bias.[22–27]

Any disagreements were resolved through discussion until consensus was reached.

Results

Study selection

A total of 185 studies were initially identified for analysis. Screening of the abstracts and titles excluded 99 studies. Twenty-one studies underwent full-text analysis.[14,17,20–38] Of these, 11 were excluded because they did not meet the predefined inclusion criteria (Table 5).[17,28–33,35–38] The main reasons for exclusion were non-randomized study design (8 studies[28–32,34,35,37]) and studies involving permanent teeth (3 studies[17,33,38]).

Table 5. Excluded studies and the reasons for their exclusion

Study	Reason for exclusion
Zhegova at al. 2014[28]	non-randomized clinical study
Jacobson and Asgari 2008[29]	non-randomized clinical study
Hossain et al. 2002[30]	non-randomized clinical study
Korkut et al. 2018[17]	study conducted on permanent teeth
Kornblit et al. 2008[31]	non-randomized clinical study
Guler et al. 2014[32]	non-randomized clinical study
Eren et al. 2013[33]	study conducted on permanent teeth
Kato et al. 2003[34]	non-randomized clinical study
Genovese et al. 2008[35]	non-randomized clinical study
Duruk et al. 2022[37]	non-randomized clinical study
Vaddamanu et al. 2022[38]	study conducted on permanent teeth

General characteristics of the included studies

Ten studies were included.[14,20–27,36] Table 6 provides an overview of the articles that met the inclusion criteria. Four studies were in vitro laboratory investigations[14,23,24,27] and 6 were in vivo clinical studies.[20–22,25,26,36] No studies have comprehensively translated the results of laboratory investigations into clinical applications.

All 10 eligible studies were randomized. Four were conducted on extracted deciduous teeth,[14,23,24,27] whereas 6 were clinical trials involving children.[20–22,25,26,36] In 6 studies, the Er:YAG laser was used,[20–22,24,26,27] while 4 studies used the Er,Cr:YSGG laser.[14,23,25,36] None of the publications directly compared the 2 laser systems (Er:YAG and Er,Cr:YSGG). All studies included a control group. Among the in vitro studies, the smallest sample consisted of 48 teeth[24] and the largest of 100 extracted teeth.[23] The remaining in vitro studies included 50 teeth[14] and 80 teeth.[27] Among the in vivo studies, the smallest sample consisted of 50 teeth,[25] while the largest included 84 teeth.[20] The remaining studies included 80 teeth,[21,26] 60 teeth[36] and 70 teeth.[22]

All included studies compared cavity preparation in deciduous teeth using Er:YAG or Er,Cr:YSGG lasers with conventional rotary instrumentation. The studies evaluated the effectiveness and efficiency of the use of the Er:YAG laser in the removal of caries in deciduous teeth,[20–22,25,26,36] microleakage after cavity preparation with the Er,Cr:YSGG laser,[23] sealing of the lacquer after the use of the Er,Cr:YSGG laser,[14] and the acceptance of laser cavity preparation by pediatric patients.[21,22,26,36] The reported results indicate that the use of the Er:YAG laser for the preparation of deciduous teeth was less effective than, or comparable to, the conventional method for deep cavity preparation, whereas caries removal from the side walls of the cavity was more effectively achieved using the conventional method.[20] Analysis of pain perception showed that most children experienced no pain during laser cavity preparation[8,21,22,25,26,36] and children remained calmer during treatment, despite procedures taking more than twice as long as conventional rotary treatment.[21] Laser-assisted cavity preparation was reported to be more comfortable for children.[22,25,36] The use of erbium lasers for cavity preparation in deciduous teeth does not eliminate the need for enamel etching with 37% orthophosphoric acid.[14,24]

Characteristics of individual studies in terms of laser settings

Table 7 summarizes the laser settings used in the included studies. Information extracted from the articles included laser wavelength and the operating parameters, namely power, frequency, fluence, pulse duration, and the amount of air-water spray.

In 7 publications, the fluence values were not reported.[14,22,24–27,36] In 2 studies, fluence values ranged from 39 J/cm^2 to 55.5 J/cm^2, although separate values for enamel

and dentin were not provided. In only 1 study were fluence values reported separately for enamel and dentin, ranging from 17.8 J/cm^2 to 26.8 J/cm^2 for dentin and from 44.6 J/cm^2 to 53.6 J/cm^2 for enamel.[23] The laser power was not reported in 8 studies.[20–23,24,26,27,36] In the remaining studies, the reported power ranged from 2.5 W to 6 W.[14,25] Pulse energy was reported in 5 studies[20–22,24,27] and ranged from 50 mJ to 700 mJ. Frequency was provided in all studies and ranged from 2 Hz,[24] through 4 Hz,[20] 10 Hz,[21] and 20 Hz,[14,23] up to 25 Hz.[25] Pulse duration was not reported in 5 studies.[21,22,24,26,36] Two publications noted pulse durations ranging from 140 µs to 200 µs,[14,23] one study reported a pulse duration of 100 µs,[20] and another reported 140 µs.[34,39] The study by Wang et al. described the use of the microsecond pulsing laser (MSP) mode without providing its numerical value.[27] Information regarding air-water spray was reported in all studies; however, different units were used. Water spray was expressed either as percentages, ranging from 60% to 85%,[14,23,25,27] or as flow rates, ranging from 5 mL/min to 6 mL/min.[20,24] Air cooling was determined in only 4 studies,[14,23,25,27] with values of 40%, 55% and 90%. The remaining 6 publications did not report air cooling settings.[20–22,24,26,36]

Characteristics of the laser applicators, types of primary teeth, and treated surfaces

The characteristics of the laser applicators, types of primary teeth treated, and treated surfaces are presented in Table 8.

The type of applicator used was not specified in 6 out of 10 studies; neither its shape nor its length was given.[20–22,25,26,36] The remaining 4 publications specified the applicator used: a G6tip sapphire tip[23]; a 2051 handpiece[24]; a 6-mm long sapphire tip[14]; and optical fiber with an R14 handle.[27] In 4 cases,[22,24,26,36] the diameter of the applicator was not reported. The remaining 6 tests were carried out with the use of applicators with a diameter of 600 µm,[14,23,25,27] 800 µm[21] or 900 µm.[20] Only 3 publications reported the angle of the laser beam during operation.[21,25,27] None of the included studies described the operator's hand movements during laser cavity preparation. One publication did not specify which primary teeth were treated with the laser.[25] The remaining 9 studies identified the treated teeth as deciduous molars,[14,20,22,26,27,36] canines[23,24] and incisors.[21] Four studies[20,21,23,25] specified the treated tooth surfaces: occlusal surfaces[20,25]; Black's Class V cavities[23]; and Black's Class III and IV cavities.[21] Six studies did not report the treated areas.[14,22,24,26,27,36] In 7 cases, the laser was operated in a non-contact mode.[14,20,21,23–25,27] However, the distance between the applicator and the target tissue varied. Four studies reported a working distance of 1 mm,[14,21,23,27] 1 study did not report the value,[6] one noted a distance of 17 mm,[24] and another – of 7 mm.[20]

Discussion

The presented results demonstrate the small number of published articles investigating the use of Er:YAG and Er,Cr:YSGG lasers in laboratory and clinical studies for the preparation of hard tissues in deciduous teeth in children, as well as the complete lack of similar systematic reviews on this topic. In addition, most of the published articles do not provide complete data on the laser operating parameters, including energy, power, frequency, applicator type and diameter, and the amount of air-water spray. Furthermore, they do not distinguish between the parameters used for individual hard tissues, namely enamel and dentin.

With regard to frequency, considerable variation was observed, ranging from 2 Hz[4] to 25 Hz.[6] A frequency of 20 Hz[14,23] was used in 2 studies, whereas 25 Hz was reported in 1 publication.[25] None of the researchers differentiated the frequency according to whether the laser was used on enamel or dentin. In the studies included in this review, the frequency values used with the Er,Cr:YSGG laser ranged from 20 Hz to 25 Hz,[14,23,25] whereas those used with the Er:YAG laser ranged from 2 Hz to 10 Hz.[20,21,24] None of the analyzed studies explained why the frequencies used with the Er,Cr:YSGG laser were considerably higher than those used with the Er:YAG laser. Likewise, no such relationship has been described in other published studies.[6,7,17,33] When preparing hard dental tissues, frequency appears to be an important parameter. The speed of the Er:YAG and Er,Cr:YSGG[35,40–49] lasers depends not only on the laser parameters and the operator's experience but also on the chemical composition of the hard dental tissues (enamel and dentin). Enamel consists of 95% of hydroxyapatite, 4% water and 1% collagen fibers, whereas dentin contains approx. 70% hydroxyapatite, 20% collagen fibers and 10% water.[44] Carious hard tissues contain more water than healthy tissues, which means that laser processing of dentin is faster than that of enamel, and diseased tissue is removed more easily than healthy tissue. Therefore, careful control of the laser parameters during cavity preparation is essential to comply with the principles of MID. During enamel preparation, higher frequency settings than during dentin preparation may be appropriate, particularly near the intraventricular wall.[31,46]

Similarly, fluence was not reported in 2 studies.[24,25] When each study is analyzed individually, it is not possible to determine which laser parameters were used for specific tooth surfaces or which parameters were applied to enamel and dentin during caries removal. One publication reported the fluence, applicator type and diameter, use of the non-contact mode at a distance of 1 mm from the target area, the type of tooth treated (canines), and the cavity type (Class V).[23] It did not describe the treated surface or the operator's movements during the procedure.[23] The study by Rossi et al. appears to be one of the most comprehensive among the included studies and provides the most useful information for determining appropriate

Table 6. Characteristics of studies that fulfilled the eligibility criteria

Study	Laser type and wavelength [nm]	Study groups	Control group	Type of the study
Valério et al. 2016[20]	Er:YAG 2,940	42 children (84 primary molars) divided into 2 groups: • Er:YAG laser group • bur preparation group	YES	in vivo
Rossi et al. 2008[23]	Er,Cr:YSGG 2,780	100 teeth divided into 10 groups: • groups 1 and 2 represented cavities prepared by a diamond bur • groups 3–10 represented cavities prepared with the Er,Cr:YSGG laser	YES	in vitro
Liu et al. 2006[21]	Er:YAG 2,940	40 children (80 teeth) divided into 2 groups: • Er:YAG laser group • bur preparation group	YES	in vivo
Johar et al. 2019[25]	Er,Cr:YSGG 2,780	25 children (50 teeth) divided into 2 groups: • group A – caries removal using the Er,Cr:YSGG laser • group B – caries removal using the air rotor handpiece	YES	in vivo
Wanderley et al. 2005[24]	Er:YAG 2,940	48 primary canines divided into 4 groups: • control (G1) + 35% phosphoric acid • 3 groups irradiated with different Er:YAG laser energies: – 60 mJ/2 Hz (G2) + 35% phosphoric acid – 80 mJ/2 Hz (G3) + 35% phosphoric acid – 100 mJ/2 Hz (G4) + 35% phosphoric acid	YES	in vitro
Sungurtekin et al. 2010[14]	Er,Cr:YSGG 2,780	50 mandibular primary molars divided into 5 groups: • 35% orthophosphoric acid etching • 2.5 W Er,Cr:YSGG laser irradiation • 3.5 W Er,Cr:YSGG laser irradiation • 2.5 W Er,Cr:YSGG laser irradiation + 35% orthophosphoric acid etching • 3.5 W Er,Cr:YSGG laser irradiation + 35% orthophosphoric acid etching	YES	in vitro
Alia et al. 2020[36]	Er,Cr:YSGG 2,780	thirty 6–12-year-old children with 60 carious molars divided into 2 groups: • laser group • bur preparation group	YES	in vivo
Abdrabuh et al. 2023[22]	Er:YAG 2,940	35 healthy children with 70 carious primary molars divided into 2 groups: • laser group • bur preparation group	YES	in vivo
Felemban et al. 2024[26]	Er:YAG 2,940	forty 9–12-year-old children with 80 carious primary molars divided into 2 groups: • laser caries removal group • conventional rotary treatment group	YES	in vivo
Wang et al. 2020[27]	Er:YAG 2,940	freshly extracted intact 80 primary molars divided into 4 groups based on the surface treatment: • control group • etchant group • energy group • frequency group	YES	in vitro

SEM – scanning electron microscopy.

Objectives	Results and conclusions
To evaluate the effectiveness and efficiency of the Er:YAG laser for caries removal in primary molars by assessing microbiological changes in dentin and clinical restoration outcomes after 1 year in 29 children.	The Er:YAG laser was less effective than bur preparation for caries removal at the pulpal wall of a deciduous molar. In the surrounding walls, bur preparation was the more effective method. Regardless of the method employed, similar amounts of *S. mutans* and *Lactobacillus* spp. were detected in the affected dentin of the pulpal wall. The Er:YAG laser and bur preparation were equally effective for caries removal from the pulpal wall; however, bur preparation was more effective on the surrounding cavity walls.
To evaluate microleakage following cavity preparation in primary teeth using an Er,Cr:YSGG laser or a high-speed drill in combination with conventional and resin-modified glass ionomer cements.	The lowest degree of dye penetration was observed in cavities prepared with the Er,Cr:YSGG laser using energy densities of 44.6 J/cm^2 (2.5 W) and 53.6 J/cm^2 (3 W) for enamel and 17.8 J/cm^2 (1 W) and 26.8 J/cm^2 (1.5 W) for dentin. No significant differences were found between cavities prepared with the Er,Cr:YSGG laser and those prepared using an air turbine.
To evaluate the clinical efficiency and patient acceptance during cavity preparation in children by directly comparing Er:YAG laser preparation with conventional mechanical preparation of caries using a split-mouth design.	Pain assessment showed that 82.5% of children experienced no pain during laser preparation, whereas greater body and head movement occurred during conventional preparation. Although Er:YAG laser preparation was 2.35 times longer than conventional preparation, 92% of children preferred laser treatment. Laser preparation appears to be a suitable option for anxious children because it causes less pain while maintaining acceptable clinical efficiency. Children experienced less pain during laser cavity preparation than during a conventional mechanical preparation.
To evaluate and compare caries removal using the Er,Cr:YSGG laser and a conventional air rotor handpiece in primary teeth.	Children experienced less pain during caries removal with the Er,Cr:YSGG laser than with the air rotor handpiece. Although treatment time was longer in the laser group, complete removal of the caries detector dye was achieved in both groups, indicating that both methods were effective for caries removal. Children reported greater comfort during laser treatment.
To assess, in vitro, the influence of Er:YAG laser energy on the shear bond strength of a total-etch adhesive system to laser-irradiated primary enamel and to evaluate the morphology of laser-ablated surfaces using SEM.	Er:YAG laser ablation of primary enamel before the adhesive protocol influenced shear bond strength. Bond strengths obtained after irradiation with 60 mJ and 80 mJ were significantly higher than those of the acid-etched control group. No significant difference was found between the control group and specimens irradiated with 100 mJ. SEM analysis demonstrated progressively rougher enamel surfaces, regardless of acid etching. The findings suggest that Er:YAG laser irradiation may be an effective method for preparing primary enamel before adhesive restorative procedures.
To evaluate the effect of Er,Cr:YSGG laser pretreatment alone or in combination with acid etching on the marginal integrity of a resin-based fissure sealant in primary teeth.	Microleakage scores in the acid-etching group were significantly lower than those in the laser-only groups, whereas no statistically significant differences were observed between groups 4 (2.5 W laser etching + acid etching) and 5 (3.5 W laser etching + acid etching). Er,Cr:YSGG laser etching does not eliminate the need for acid etching.
To assess and compare pain sensitivity, anxiety and willingness to undergo cavity preparation in children using the Er,Cr:YSGG laser (2,780 nm) and the conventional rotary method.	The air rotor group showed a significantly higher pulse rate compared to the baseline, while the laser group did not exhibit any significant differences in pulse rate. Pain scores were slightly higher in the air rotor group than in the laser group, and 57% of children preferred the Er,Cr:YSGG laser for future cavity preparation. During cavity preparation, the Er,Cr:YSGG laser appears to be more effective and better accepted by children because it induces less anxiety and causes less pain in comparison to the air rotor.
To evaluate and compare children's anxiety levels and pain perception during cavity preparation using an Er:YAG laser vs. conventional rotary instrumentation.	The mean participant age was 9.4 ±1.29 years, and 51.4% of the participants were boys. Baseline pulse rates did not differ significantly between the laser and conventional treatment groups. During treatment, pulse rates were significantly higher with conventional rotary instrumentation than with the Er:YAG laser. Significantly fewer children requested local anesthesia during laser treatment. The use of the Er:YAG laser for caries removal reduces pain and anxiety, decreases the need for local anesthesia, and helps children remain more relaxed during treatment.
To evaluate and compare the effectiveness of cavity preparation using an Er:YAG laser and conventional rotary instrumentation in children, with particular emphasis on the quality of the bond interface.	SEM analysis of the adhesive interface demonstrated no significant differences between restorations placed after bur preparation and those placed after Er:YAG laser preparation. Both methods showed favorable outcomes. After 1 year, no significant differences in adhesive interface quality were observed.
To investigate the effect of Er:YAG laser pretreatment on dentin structure and the shear bond strength of deciduous teeth.	SEM demonstrated that the surface structure of dentin slices underwent alterations following acid etching and Er:YAG laser pretreatment. Laser settings of 50–200 mJ and 5–20 Hz resulted in open dentinal tubules, whereas deviations from this range caused cracks and breakdown of intertubular dentin structure. Shear bond strength increased significantly following laser pretreatment compared with the control group ($p < 0.05$). Enhanced shear strength was observed within the same energy and frequency range as tubule opening, although the difference was not significant ($p > 0.05$). The predominant mode of interface failure was adhesive, followed by mixed and resin cohesive failures. Er:YAG laser pretreatment effectively opens dentinal tubules, avoids smear layer formation, and improves the bond strength between primary dentin and resin composites.

Table 7. Characteristics of the laser settings

Study	Laser type	Energy density (fluence) [J/cm²]	Power [W]	Pulse energy [mJ]	Frequency [Hz]	Water spray	Air cooling [%]	Pulse duration [µs]
Valério et al.[20]	Er:YAG	39	NA	250	4	6 mL/min	NA	100
Rossi et al.[23]	Er,Cr:YSGG	Enamel preparation: • 44.6 • 53.6 Dentin preparation: • 17.8 • 26.8	Enamel preparation: • 2.5 • 3.0 Dentin preparation: • 1.0 • 1.5	NA	20	65%	55	140–200
Liu et al.[21]	Er:YAG	55.5	NA	700	10	24 mL/min	NA	NA
Johar et al.[25]	Er,Cr:YSGG	NA	6	NA	25	60%	40	140
Wanderley et al.[24]	Er:YAG	NA	NA	60 80 100	2	5 mL/min	NA	NA
Sungurtekin et al.[14]	Er,Cr:YSGG	NA	2.5 3.5	NA	20	80% 85%	90 90	140–200
Alia et al.[36]	Er,Cr:YSGG	NA	NA	NA	NA	NA	NA	NA
Abdrabuh et al.[22]	Er:YAG	NA	NA	100–200	20	NA	NA	NA
Felemban et al.[26]	Er:YAG	NA	NA	NA	NA	NA	NA	NA
Wang et al.[27]	Er:YAG	NA	NA	50–200	5–20	60%	40	NA

NA – information not available.

Table 8. Characteristics of the laser applicators, types of primary teeth and treated surfaces

Study	Applicator type	Applicator diameter [µm]	Setting angle	Operating mode	Operator movement	Type of primary teeth	Treated surface
Valério et al.[20]	NA	900	NA	non-contact mode (focal distance: 7 mm (R02))	NA	molars	occlusal
Rossi et al.[23]	G6tip sapphire tip	600	NA	non-contact mode (1 mm from the target area)	NA	canines	Black's Class V
Liu et al.[21]	NA	800	90°	non-contact mode (1 mm from the target area)	NA	incisors	Black's Classes III and IV
Johar et al.[25]	NA	600	90°	non-contact mode	NA	NA	Black's Class I
Wanderley et al.[24]	2051 handpiece	NA	NA	non-contact mode (17 mm from the target area)	NA	canines	NA
Sungurtekin et al.[14]	6-mm long sapphire tip	600	NA	non-contact mode (1 mm away from the target area)	NA	molars	NA
Alia et al.[36]	NA	NA	NA	NA	NA	molars	NA
Abdrabuh et al.[22]	NA	NA	NA	NA	NA	molars	NA
Felemban et al.[26]	NA	NA	NA	NA	NA	molars	NA
Wang et al.[27]	optical fiber R14 handle	600	vertical to the surface	non-contact mode (1 mm from the target area)	NA	molars	NA

Er,Cr:YSGG laser settings for enamel and dentin preparation in primary teeth with Class V cavities.[23]

After comparing the studies on deciduous teeth included in this review with studies on permanent teeth that were excluded from the analysis because they were outside the scope of this review, a considerable lack of consistency in reporting laser operating parameters was observed. Furthermore, the available studies do not provide tissue-specific parameters for enamel and dentin. Apart from reporting the laser wavelength, the published studies generally fail to provide essential information, including the type of handpiece, applicator type, applicator diameter, operating mode (contact or non-contact), working distance, angle of laser application, operator movement, type of tissue treated (enamel or dentin), type of teeth treated (primary, immature permanent, or mature permanent), treated tooth surface, and laser operating parameters, including pulse duration, frequency, pulse energy, power, fluence, water spray, and air cooling. Among the analyzed studies, wavelength, frequency and water spray were the only parameters consistently reported. However, water spray was expressed either as mL/min or as percentages, making direct comparison impossible because no validated method exists for converting percentage values into water flow rates.

Two of the reviewed papers[20,25] investigated cavity preparation on the occlusal surfaces of deciduous molars. The first paper evaluated the Er:YAG laser,[20] whereas the second investigated the Er,Cr:YSGG laser.[25] The reported frequencies differed substantially: 4 Hz[20] vs. 25 Hz.[25] In the first study, the fluence was 39 J/cm^2, and in the second study, no fluence value was provided. The first paper also reported pulse energy and pulse duration, making it possible to establish baseline data for Er:YAG laser preparation of occlusal surfaces in primary teeth. However, the authors did not specify whether these parameters were used for enamel or dentin preparation, which is an important limitation. The provided information is insufficient to establish basic Er,Cr:YSGG laser parameters for enamel and dentin preparation on occlusal surface of deciduous teeth.[25] Valério et al.[20] used a 900-µm applicator in a non-contact mode with a focal distance of 7 mm, whereas Johar et al.[25] used a 600-µm applicator in a non-contact mode but did not specify either the working distance or the applicator type. Neither study indicated whether the reported parameters referred to enamel or dentin preparation.

Following the analysis of the reported laser settings, although 7 publications reported the applicator diameter,[8,14,20,21,23–25] only 4 specified the applicator type.[14,23,24,27] None reported the angle between the applicator and the treated tissue, and none described the operator's hand movements. All studies used the non-contact technique, reporting working distances of 1 mm,[14,21,23] 7 mm,[20] 17 mm,[24] or not specifying the distance from the treated surface.[25] However, the rationale for selecting these distances was never explained.

The included studies also provide only general information regarding applicator type and diameter. In contrast, several studies involving permanent teeth, although excluded from this review, described more detailed treatment protocols. Among these, Kato et al. described laser cavity preparation in both permanent and deciduous teeth using the Er:YAG laser.[34] They reported pulse duration, applicator type and diameter, as well as separate laser parameters for enamel and dentin. However, they did not describe the operator's movements or distinguish between laser settings used for deciduous and permanent teeth. Consequently, their findings cannot be used to establish standardized laser parameters for either dentition.[34]

Another excluded study by Eren et al. assessed pain during Er,Cr:YSGG laser treatment of permanent teeth in children.[33] The authors reported different air-water spray settings for enamel (85% air and 85% water) and dentin (65% air and 55% water). They also described the head and the applicator used and were the only authors to report the operator's movements: "During preparation, the sapphire tip (600 µm) was moved carefully back and forth and up and down to remove caries." The authors compared treatment duration with conventional method in first permanent molars of children aged 7–10 years. Laser preparation required an average of 7.4 min, whereas mechanical preparation required approx. 3.7 min.[33] Liu et al. likewise compared treatment times in primary teeth and found that Er:YAG laser preparation required approx. 2.35 times longer than conventional methods.[21]

Overall, laser preparation requires nearly twice as much time as conventional rotary instrumentation. However, pain is one of the most important factors contributing to uncooperative behavior in pediatric patients. The reviewed studies indicate that approx. 70% of children preferred laser preparation, compared with 30% who preferred conventional treatment. Although laser preparation is slower, children cooperate more readily, remain calmer during treatment, and frequently choose laser treatment again during subsequent visits.[21,25,33] These advantages make laser-assisted treatment less traumatic and more acceptable for pediatric patients.

The only conclusion resulting from the presented analysis is that studies investigating Er:YAG and Er,Cr:YSGG lasers have been published with similar frequency.

The main common finding to all published studies is that erbium lasers provide advantages because they operate without direct contact with the tissues of the prepared tooth and without vibration.[21,24,33,34,39,40] Approximately 90% of children accept laser dental treatment,[33] while patients aged 10 years or older generally show no preference between laser and conventional treatment.[34,36] The reduction in turbine noise appears to be particularly important for younger children, taking into account comfort during work.[21,24,33,34,39–41] In most of the presented cases, local anesthesia was not required during laser treatment.[14,20,21,23–25,33,34,39,40] Laser cavity preparation enables minimally invasive and selective removal of infected carious tissues.[34,39,42,43] The use of laser techniques in hard tissues produces little or no thermal effect on the dental pulp.[34,39,44,45] Dentin prepared with the Er:YAG laser is free of a smear layer, and the dentinal tubules remain open and visible.[34,43,45,47–49]

Removal of carious tissue from the side walls of the cavities is more difficult with laser preparation than with conventional methods because the laser applicator does not function in the same manner as a drill. In contrast, preparation of infected dentin on the intraventricular wall is comparable with conventional mechanical preparation.[20]

The use of laser technology represents a new philosophy of treatment for both dentists and patients. Laser-assisted preparation enables young patients to undergo treatment in a more comfortable and less stressful environment.

The limited amount of published information regarding laser treatment of primary teeth justifies further research in this area and highlights the need to establish standardized treatment protocols for laser preparation of hard tissues in primary teeth.

Based on the available evidence, there is a clear need for further laboratory and clinical studies. Future publications should consistently report the following information: laser wavelength; head type; applicator type and diameter; operating mode (contact or non-contact); working distance; angle of laser application; operator movement; tissue

treated (enamel or dentin); tooth type (primary, immature permanent, or mature permanent); treated tooth surface; pulse duration; frequency; pulse energy; power; fluence; water spray; and air cooling.

The use of the Er,Cr:YSGG laser for cavity preparation in deciduous teeth may be more effective than conventional rotary instruments in reducing anxiety and pain in young patients. Therefore, the Er,Cr:YSGG laser may represent an alternative to conservative treatment in children.[36] The use of the Er:YAG laser for cavity preparation is also associated with minimal pain and discomfort, thereby reducing the need for local anesthesia and providing a more comfortable treatment option for children than the conventional method.[22] Additionally, erbium laser technology may enable more precise cavity preparation than traditional methods, resulting in improved adaptation and bonding of the filling material. This may contribute to greater restoration longevity and a lower risk of recurrent caries in deciduous teeth.[26] Following Er:YAG laser irradiation of dentin, dentinal tubules may undergo morphological changes. Laser energy can induce partial sealing or closure of the dentinal tubules, thereby reducing their diameter. This sealing effect can help reduce dentin hypersensitivity and improve the bond strength of restorative materials to the treated surface.[27,37]

When Er:YAG lasers are used for cavity preparation in primary teeth, specific laser parameters influence treatment effectiveness. Commonly recommended parameters include:

- Pulse duration: Short pulse durations (approx. 50–100 μs) are preferred for precise ablation with minimal thermal damage to the surrounding tissues;
- Energy density: Fluence should be optimized (approx. 20–100 mJ/cm^2) to ensure efficient ablation while preventing overheating of the tooth structure;
- Repetition rate: The repetition rate may vary (2–20 Hz), depending on the desired ablation efficiency and thermal effects. Higher repetition rates may accelerate cavity preparation but require careful monitoring to prevent tissue damage;
- Water spray or air-water cooling: The use of water spray or air-water cooling helps dissipate heat generated during laser ablation and reduces the risk of thermal injury to the dental pulp.

Although laser-assisted cavity preparation in deciduous teeth shows considerable promise, additional studies are required to establish the optimal irradiation parameters for clinical use.[38]

The development of erbium family lasers in pediatric dentistry, particularly for the treatment of deciduous teeth, is likely to progress in several directions:

1. Improved treatment efficiency: Continued advances in laser technology and optimization of operating parameters may improve precision, shorten treatment time and enhance clinical outcomes during caries removal, cavity preparation and soft tissue procedures in deciduous teeth;
2. Minimally invasive treatment: Erbium lasers facilitate minimally invasive procedures that preserve healthy tooth structure, reduce the need for anesthesia, and improve patient comfort during dental treatment, making them particularly advantageous in pediatric dentistry;
3. Preventive applications: Further research may explore the use of erbium lasers for preventive procedures, such as enamel conditioning, fissure sealant application and surface modification to enhance the resistance of deciduous teeth to caries;
4. Treatment of patients with special needs: Erbium lasers may provide a gentle and efficient alternative for managing dental conditions in children who have difficulty tolerating conventional dental treatments.
5. Combination therapies: Combining erbium laser treatment with other dental modalities, such as fluoride therapy, sealants or antimicrobial agents, may further improve the effectiveness of caries prevention and management in deciduous teeth.

The future application of erbium family lasers in pediatric dentistry appears promising, offering innovative approaches to the treatment of deciduous teeth with improved precision, minimal discomfort and reduced anxiety for young patients. By addressing important challenges in pediatric dental care, including fear of dental procedures, pain management and patient cooperation, erbium lasers have the potential to revolutionize the treatment experience and improve oral health outcomes in children.

Overall, the ongoing development and clinical implementation of erbium laser technology in pediatric dentistry represent a promising direction toward safer, more effective and more patient-friendly dental care for children.

Limitations

One limitation of this systematic review is the limited number of high-quality studies available on the use of erbium family lasers for cavity preparation in deciduous teeth. Consequently, it is difficult to draw definitive conclusions because of the limited amount of robust scientific evidence. The available studies are generally small, employ different methodologies, and often report inconsistent outcomes, making comprehensive evaluation of the effectiveness and safety of erbium lasers challenging.

Several additional limitations should be considered when interpreting studies on laser treatment of carious lesions in deciduous teeth:

- Sample size and diversity: The limited availability of pediatric patients with carious lesions may restrict both sample size and population diversity, affecting the statistical power and limiting the generalizability of the findings;
- Ethical considerations: Clinical studies involving pediatric populations require strict ethical standards, including informed parental consent, child assent when appropriate, and careful attention to patient comfort and safety throughout treatment;

- Long-term follow-up: Assessment of long-term clinical outcomes is challenging due to the transient nature of primary dentition and the potential loss of follow-up over time;
- Variability in tooth morphology: Deciduous teeth exhibit diverse anatomical features, making it difficult to standardize laser treatment protocols across different tooth types and stages of dental development.

Research involving deciduous teeth is challenging because of the limited availability of suitable teeth and their eventual replacement by permanent dentition. Consequently, studies involving primary teeth often include smaller sample sizes than studies conducted on permanent teeth. Researchers may face difficulties in recruiting sufficiently large study populations because of ethical considerations, patient age and the natural shedding process of these teeth.

Despite the potential limitations in sample size, studies involving deciduous teeth remain essential for improving our understanding of pediatric oral health and for developing appropriate treatment strategies tailored to this age group. These limitations should therefore be considered when designing and interpreting research involving deciduous teeth to make informed decisions regarding pediatric dental care.

Conclusions

Based on the available evidence, it is currently not possible to establish optimal Er:YAG and Er,Cr:YSGG laser parameters for the preparation of carious enamel and dentin in deciduous teeth. Analysis of the included studies demonstrates that only limited information is available regarding the laser operating parameters used during treatment. Individual data values are missing to determine the final laser settings. The information reported in the available studies is incomplete and insufficient to allow reliable assessment of the physical laser parameters.

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

Efficacy, safety, and health-related quality of life data of hyaluronic acid for the treatment of interdental papilla deficiency: A systematic review of randomized controlled trials

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Conflict of interest
None declared

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Abstract

The efficacy of hyaluronic acid (HA) in the treatment of interdental papilla (IDP) deficiency is well documented. This systematic review sought to evaluate clinical studies reporting efficacy, safety, and health-related quality of life (HRQoL) data for HA used in the treatment of IDP deficiency. This review was conducted and reported in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology. A comprehensive search of electronic databases was conducted for published randomized controlled trials (RCTs) from their inception until March 21, 2024. A total of 9 studies met the inclusion criteria. The sample sizes in the included RCTs ranged from 10 to 31 participants, with the mean patient age between 26.7 and 46.1 years, predominantly females. Of the 9 eligible studies, 6 assessed the use of HA alone, while 3 investigated the effects of HA in combination with other therapies. The most common outcome measures assessed black triangle dimensions (i.e., area, height or width) followed by probing depth (PD). Based on the limited published studies, this review suggests that HA treatment (whether alone or in combination) may offer therapeutic benefits for patients with IDP defects. However, none of the included studies assessed the safety of HA and its effect on HRQoL. There is a need for further research on the long-term efficacy and safety of HA treatment, and its influence on HRQoL.

Keywords: periodontal disease, hyaluronic acid, efficacy, health-related quality of life, interdental papilla deficiency

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Highlights

- Hyaluronic acid (HA) appears to be an effective and minimally invasive treatment option for restoring interdental papilla (IDP) deficiency.
- Most included RCTs reported improvement in black triangle dimensions following HA treatment.
- Combination therapies may provide additional benefits compared with HA alone.
- Evidence regarding HA safety remains limited.
- None of the included studies evaluated health-related quality-of-life outcomes.

Introduction

The interdental papilla (IDP) represents the visible anatomical part of the gingival tissue that extends from the interproximal alveolar crest to the contact point.[1] Although the papilla is a small anatomical structure, it has a significant functional and esthetic value.[1,2] The deficiency or loss of IDP leads to an open gingival embrasure space, termed a "black triangle", which adversely affects esthetics, increases the retention of food debris and contributes to phonetic problems.[2] Therefore, the preservation and restoration of a deficient or lost IDP are essential. Nevertheless, the reconstruction of a lost IDP presents a significant challenge, often yielding unpredictable long-term outcomes, and remains a topic of debate and further investigation in the periodontal field.[3]

Several surgical methods, including pedicle flaps, semilunar coronally repositioned flaps, and regenerative procedures with connective tissue and bone grafts, have been proposed and used either alone or in combination to treat IDP loss. However, the invasiveness and uncertain effectiveness of surgical procedures, together with the anatomical location of IDP and its poor blood supply, still pose a major challenge.[4,5] Considering the potential limitations associated with surgical procedures, several non-surgical options (i.e., hemolaser therapy, orthodontic treatment, restorative treatment, and the injection of fillers and gingival tissue volumizers) have been reported to offer better therapeutic benefits; however, their use in routine dental practice remains limited.[3,6]

Hyaluronic acid (HA), a naturally occurring polysaccharide, is present within the extracellular matrix (ECM) of various body tissues, including periodontal tissues.[7] Hyaluronic acid has been demonstrated to play a significant role in regulating various biological processes, including anti-inflammatory responses, wound healing and tissue regeneration.[8] Several controlled trials have reported the clinical efficacy of HA in the treatment of IDP deficiency.[1,5,8,9–14] Moreover, evidence from the published systematic reviews indicates that HA treatment is efficacious in patients with IDP deficiency.[3,6,7,15,16] However, none of the published reviews have reported on the health-related quality of life (HRQoL) of IDP-deficient patients treated with HA. Therefore, the aim of the current systematic review was to evaluate the efficacy and safety of HA use, and its effect on HRQoL in patients with IDP deficiency.

Methods

Study design

This systematic review was conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[17] A protocol for the systematic review was developed and registered in the International Prospective Register of Systematic Reviews (PROSPERO) database (CRD42024547932).

Literature search

Electronic databases (MEDLINE, MEDLINE In-Process, Embase, and the Cochrane Library) were comprehensively searched from inception to March 21, 2024, via the Ovid platform. The search strategy used to retrieve relevant studies is presented in the supplementary material, openly available in https://figshare.com at doi:10.6084/m9.figshare.26863660. A gray literature search was performed, and the bibliographies of the published systematic reviews were screened. Additionally, a manual search of unpublished work, including theses and dissertations, was performed to identify any relevant information.

Study selection and data extraction

All retrieved citations were collated after duplicates were removed. The collated citations were screened by 2 independent reviewers (DA and SA). Initially, each citation was screened based on its title and abstract, using predefined eligibility criteria (Table 1).

Subsequently, the full-texts of potentially eligible studies were retrieved and further subjected to full-text screening, using a similar process. Any uncertainty regarding study inclusion at either stage was resolved through mutual discussion. Relevant data from the included studies were extracted by a single reviewer using a predefined MS Excel-based data extraction grid. The extracted data were thoroughly checked for transcription errors by a second reviewer.

The data extracted from the eligible studies included study information (i.e., primary author name, year of publication, country, number of deficiency sites, number of patients, age, sex, study design, intervention group, deficiency

region, and treatment duration), and outcomes of interest (i.e., black triangle area, height and width, probing depth (PD), gingival index (GI), deficient volume, papillary deficiency height, satisfaction score, plaque index (PI), papilla presence index (PPI), modified papilla index score (MPIS), distance between the papilla tip and the contact point, gingival volume, clinical attachment level/loss (CAL), bleeding on probing (BoP), radiographic alveolar bone height, esthetic appearance, pain, signs of black triangle, and papillary marginal gingival index).

Quality assessment of the included studies

The Cochrane risk-of-bias assessment tool (RoB) was used to evaluate the methodological quality of the included RCTs.[18] Two independent reviewers assessed the risk of bias for individual bias domains (i.e., selection, performance, detection, attrition, reporting, and any other biases not covered by these categories). The risk was reported as "low", "high" or "unclear". Any discrepancies between the reviewers were resolved through mutual discussion.

Table 1. Study eligibility criteria

Category	Inclusion criteria	Exclusion criteria
Population	Patients with IDP deficiency	• Healthy volunteers • Patients without IDP deficiency
Intervention	Hyaluronic acid	Interventions other than hyaluronic acid
Comparator	Any pharmacological therapy	None
Outcomes	• Efficacy • Safety • Quality of life	Outcomes other than those specified in the inclusion criteria
Study design	• RCTs • Systematic reviews and meta-analyses of RCTs/non-RCTs[a]	• Non-RCTs including comparative and non-comparative observational studies • Preclinical studies • Comments, letters, and editorials • Case reports and case series • Pharmacokinetic and economic studies
Language	English	Studies published in languages other than English
Time frame	From inception to March 21, 2024	None

IDP – interdental papilla; RCTs – randomized controlled trials.

Results

A total of 2,373 unique citations were obtained from the electronic database search. Following primary screening, 2,343 citations were excluded, as they did not meet the inclusion criteria, while 30 citations were subjected to full-text review. Of these, 5 studies were included in the systematic review. Additionally, 4 studies were identified through the bibliographic search, resulting in a total of 9 unique studies included in the final synthesis (Fig. 1).

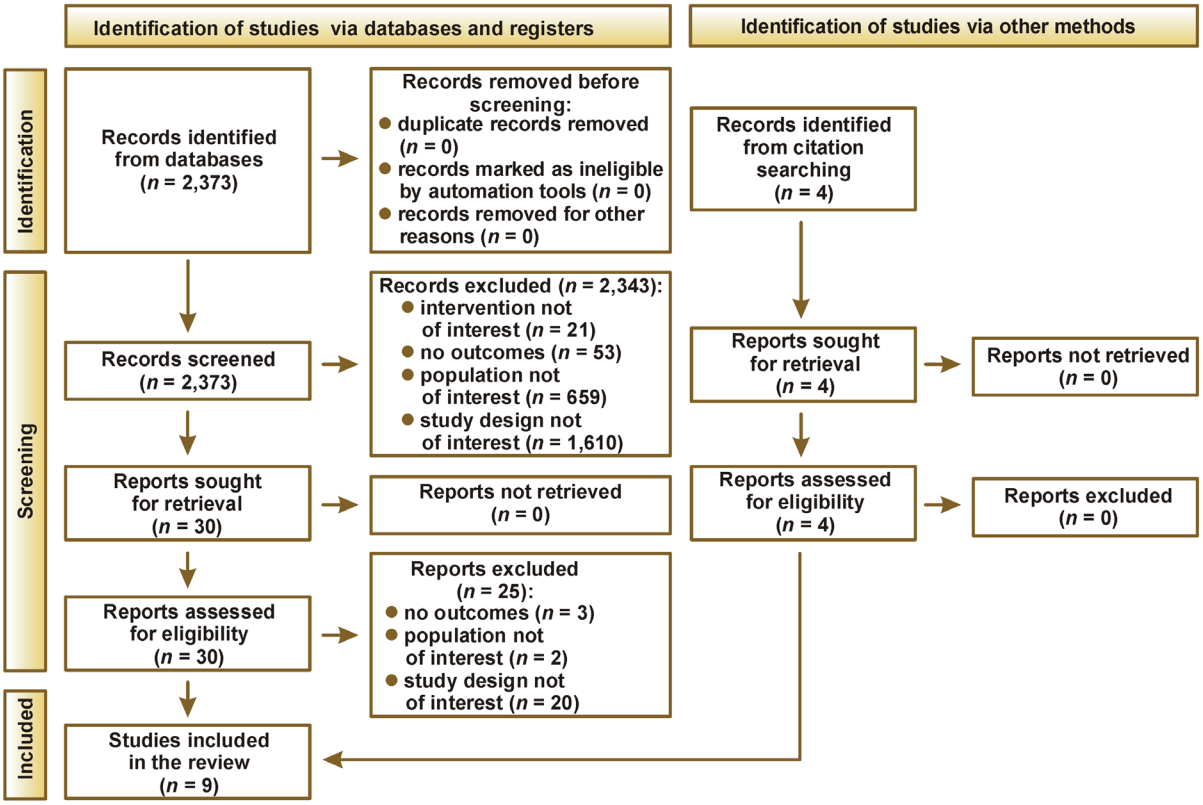


Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart for study selection

Study characteristics

The characteristics of the included studies are summarized in Table 2. All studies assessed patients with IDP deficiency. Four studies were conducted in Egypt, 2 in India, and 1 each in China, Sweden and Hungary. Of all included studies, 3 were double-blind RCTs, one was a single-blind RCT (i.e., investigator-blinded), and the remaining 5 studies did not report blinding information.

Of the 9 studies included in this review, only 2 studies explicitly specified outcomes assessed as primary and secondary outcomes. In the RCT conducted by Ni et al., the primary outcome measure was the height of each gingival papilla, while the area of each black triangle was assessed as the secondary outcome.[1] In the randomized, comparative clinical study by Fakher et al., patient satisfaction was assessed as the primary outcome measure.[5] Additionally, the evaluated secondary outcomes included changes in

Table 2. Characteristics of the included studies (*N* = 9)

Author, year	Country	Study design	Number of patients	Number of sites	Mean age (*SD*)	Female, *n* (%)	Intervention groups	Classification/ region	Tx duration
Ni, 2021[1]	China	Double-blind RCT	Total: 24	HA: 34 Saline: 34	Total: 41.3 (7.73) years	Total: 19 (90.47)	• Saline 16 mg/mL HA gel	–	6 weeks
Fakher, 2023[5]	Egypt	RCT	–	HA gel: 6 iPRF: 6 HA + iPRF: 6	Total: 20–50 years	–	• HA gel: 0.2 mL	Class I or II deficiency based on Nordland and Tarnow classification	6 weeks
Abdelkader, 2022[8]	Egypt	RCT	–	HA + carboxymethyl chitosan hydrogel: 21 HA alone: 21	Total: 20–40 years	–	• HA: 0.2 mL of 50% HA gel • HA + carboxymethyl chitosan hydrogel: 0.2 mL of 50% HA gel + 50% of chitosan gel	Class I or II deficiency in interbicuspidregion interdental space based on Nordland and Tarnow classification	–
Bertl, 2016[9]	Sweden	Double-blind	HA: 11 Saline: 11	–	HA: 26.7 (5.2) years Saline: 33.1 (6.0) years	HA: 5 (45.45) Saline: 7 (70)	• HA: 1 mL contains 16 mg of cross-linked sodium hyaluronate and 2 mg Na-hyaluronate	Deficiency in the anterior maxilla	4 weeks
Shawky, 2017[10]	Egypt	RCT	Total: 30	HA gel: 20 Radiesse: 20	Total: 25–35 years	Total: 30 (100)	• HA gel: 0.2 mL • Radiesse: 0.2 mL	–	6 months
Abdelraouf, 2019[11]	Egypt	Double-blind	HA: 5 Saline: 5	HA: 16 Saline: 14	Total: 32.55 (9.3) years	HA: 4 (80) Saline: 4 (80)	• HA: 20 mg/mL	Class I or II deficiency based on Nordland and Tarnow classification	6 weeks
Mandel, 2020[12]	Hungary	RCT	Revident: 16 Flex Barrier: 15	Revident (HA) Test: 48 Revident (HA) Untreated control: 32 Flex Barrier (HA) Test: 50 Untreated control: 30	Revident: 46.1 (12.3) years Flex Barrier: 41.8 (13.8) years	Revident: 11 (68.75) Flex Barrier: 12 (80)	• Flex Barrier: 0.1 mL per site • Revident: Tx applied using gel	Class I or II IDP loss in anterior region between canine teeth based on Nordland and Tarnow classification	Single administration
Kapoor, 2022[13]	India	RCT	Total: 15	HA: 30 Saline: 30	HA: 37.13 (8.22) years Placebo: 33.4 (7.89) years	–	• HA: 0.2 mL of 2% HA gel	Class I or II deficiency based on Nordland and Tarnow classification	6 weeks
Bal, 2023[14]	India	Single-blind RCT	HA: 10 HA + PRGF: 11	HA: 18 HA + PRGF: 18	HA: 34.63 (5.22) years HA + PRGF: 38.71 (8.4) years	–	• 0.8% of 0.2 mL HA gel • 0.8% of 0.2 mL HA gel + 0.2 mL PRGF	Class I or Class II IDP loss in the maxillary region based on Nordland and Tarnow classification	6 weeks

IDP – interdental papilla; iPRF – injectable platelet-rich fibrin; HA – hyaluronic acid; mg – milligram; mL –milliliter; PRGF – plasma rich in growth factors; RCT – randomized controlled trial; SD – standard deviation; Tx – treatment.

the surface area and height of the black triangle, clinical parameters (PD, PI and GI at the deficient papilla site), and pain levels 4 h after injection.

Three studies assessed outcomes for HA in combination with other interventions as compared to HA alone, while the remaining studies assessed outcomes for HA with different formulations (i.e., Flex Barrier, Revident) or with different comparators (i.e., physiological saline, untreated controls, placebo, or calcium hydroxylapatite). The treatment duration among the included studies ranged from a single administration to 6 months. The study population in the included RCTs ranged from 10 to 31 patients. The mean age of the patients included in the RCTs ranged from 26.7 to 46.1 years, and the participants were predominantly female. Most of the included RCTs used the Nordland and Tarnow classification[19] to stratify IDP deficiency among patients (n = 6) (Table 2).

Outcome measures

Overall, most studies reported clinical outcomes, while only 3 studies reported patient-reported satisfaction following HA treatment. Among the studies that assessed clinical outcomes, black triangle area was the most frequently reported outcome (n = 7) followed by black triangle height (n = 5), PD (n = 3), black triangle width (n = 2), GI (n = 2), and CAL (n = 2). Other clinical outcomes reported included deficient volume, PI, IDP height, pain intensity, gingival volume, BoP, MPIS, radiographic alveolar bone height, esthetic appearance, clinical signs of black triangle, papillary marginal gingival index, and PPI (Table 3).

Most of the included RCTs (n = 8) reported that HA provided therapeutic benefits to patients with IDP deficiency. Hyaluronic acid was reported to be more effective in the treatment of IDP deficiency when used in combination with other interventions than HA alone. Specifically, HA in combination with plasma-rich growth factors (PRGF) was reported to provide significant improvement in papillary deficiency height, area and volume. Similarly, when HA was used in combination with injectable carboxymethyl chitosan hydrogel, improvement was reported in multiple clinical parameters (i.e., PI, GI, PD, and the surface area and height of the black triangle), while HA in combination with injectable platelet-rich fibrin (iPRF) was reported to improve black triangle height. Two studies reported beneficial effects of HA; however, the effect was not superior to that of the comparators (i.e., calcium hydroxylapatite and physiological saline).

Another study assessed the efficacy of single injections of 2 different HA preparations (i.e., Revident and Flex Barrier). Both demonstrated clinical applicability, with Revident providing longer-lasting improvement than Flex Barrier. Three RCTs compared the efficacy of HA with that of physiological saline. While 2 studies indicated a significant reduction in black triangle dimensions following the use of HA gel as compared to saline solution, the study

Table 3. Summary of the results from the included studies (N = 9)

Author, year	Outcome	Key takeaway
Ni, 2021[1]	Mean (SD) papillary deficient height (mm) Physiological saline • Baseline: 2.99 (1.42) • 6 months: 3.12 (1.36) • 12 months: 3.26 (1.39) HA gel • Baseline: 3.25 (1.30) • 6 months: 3.45 (1.27) • 12 months: 3.53 (1.25) Mean (SD) change from the baseline Physiological saline • 6 months: 0.135 (0.39) (p = 0.199) • 12 months: 0.278 (0.45) (p = 0.006) HA gel • 6 months: 0.198 (0.34) (p = 0.011) • 12 months: 0.280 (0.38) (p = 0.001) Mean (SD) black triangle area (mm^2) Physiological saline • Baseline: 1.78 (1.31) • 6 months: 1.63 (1.26) • 12 months: 1.46 (1.26) HA gel • Baseline: 1.90 (1.37) • 6 months: 1.65 (1.32) • 12 months: 1.45 (1.16) Mean (SD) change from the baseline Physiological saline • 6 months: –0.150 (0.37) (p = 0.098) • 12 months: –0.320 (0.50) (p = 0.004) HA gel • 6 months: –0.260 (0.42) (p = 0.007) • 12 months: –0.450 (0.54) (p < 0.001)	This study suggest that the height of gingival papillae could be enhanced by injecting HA to address gingival papilla defects. Nevertheless, this effect is not better than that of a physiological saline
Fakher, 2023[5]	Mean satisfaction score HA gel • Baseline: 3.8 • 6 months: 9.1 iPRF • Baseline: 3.7 • 6 months: 7.8 HA gel + iPRF • Baseline: 3.5 • 6 months: 8.7 Mean black triangle height (mm) HA gel • Baseline: 1.73 • 3 weeks: 1.4 • 6 weeks: 1.17 • 3 months: 0.72 6 months: 0.13 iPRF • Baseline: 1.98 • 3 weeks: 1.68 • 6 weeks: 1.38 • 3 months: 1.2 • 6 months: 0.92 HA gel + iPRF • Baseline: 2.5 • 3 weeks: 2.2 • 6 weeks: 1.93 • 3 months: 1.63 • 6 months: 1.33 Mean (SD) % of change of black triangle height HA gel • 3 weeks: –17.65 • 6 weeks: –31.18 • 3 months: –57.65 • 6 months: –92.35 iPRF • 3 weeks: –15.15 • 6 weeks: –30.30 • 3 months: –39.39 • 6 months: –53.54	For gingival black triangles, iPRF and HA injections may be non-invasive alternatives

Author, year	Outcome	Key takeaway
	HA gel + iPRF • 3 weeks: –12.00 (p=0.321) • 6 weeks: –22.80 ($p = 0.067$) • 3 months: –34.80 ($p = 0.000$) • 6 months: –46.80 ($p = 0.000$)	
Abdelkader, 2022[8]	Mean (SD) plaque index HA + carboxymethyl chitosan hydrogel • Baseline: 1.43 (1.27) • 3 months: 0.29 (0.49) • 6 months: 0.0 (0.0) HA alone • Baseline: 1.43 (1.27) • 3 months: 0.57 (0.79) • 6 months: 0.14 (0.38) Mean (SD) gingival index HA + carboxymethyl chitosan hydrogel • Baseline: 1.14 (1.07) • 3 months: 0.0 (0.0) • 6 months: 0.0 (0.0) HA alone • Baseline: 1.14 (1.21) • 3 months: 0.29 (0.49) • 6 months: 0.0 (0.0) Mean (SD) probing depth (units not reported) HA + carboxymethyl chitosan hydrogel • Baseline: 3.79 (0.76) • 3 months: 3.07 (0.19) • 6 months: 2.79 (0.27) HA alone • Baseline: 3.86 (0.90) • 3 months: 3.29 (0.49) • 6 months: 3.14 (0.48) Mean (SD) black triangle height (mm) HA + carboxymethyl chitosan hydrogel • Baseline: 1.98 (0.45) • 3 months: 1.71 (0.45) • 6 months: 1.33 (0.47) HA alone • Baseline: 2.23 (0.40) • 3 months: 2.03 (0.40) • 6 months: 1.79 (0.34) Mean (SD) black triangle area (mm^2) HA + carboxymethyl chitosan hydrogel • Baseline: 0.66 (0.20) • 3 months: 0.34 (0.11) • 6 months: 0.19 (0.07) HA alone • Baseline: 0.68 (0.15) • 3 months: 0.42 (0.08) • 6 months: 0.24 (0.05) Mean (SD) satisfaction score HA + carboxymethyl chitosan hydrogel • Baseline: 3.29 (1.11) • 6 months: 8.86 (0.69) HA alone • Baseline: 4.29 (1.11) • 6 months: 9.14 (0.90)	Injectable carboxymethyl chitosan hydrogel and HA gel Tx of the black triangle of the IDP result in greater improvements in clinical results. After six months, there was no statistically significant difference in the number of black triangles between the two groups
Bertl, 2016[9]	Percentage of patients with a MPIS[a] of 2 HA • Baseline: 11 (100.0) • 3 months: 11 (100.0) • 6 months: 11 (100.0) Saline • Baseline: 10 (100.0) • 3 months: 10 (100.0) • 6 months: 10 (100.0) Mean (SD) distance between PT-CP[b] (mm) HA • Baseline: 2 (1.1) • 3 months: 1.8 (0.7) • 6 months: 1.9 (0.8)	Injecting HA near the implant-supported crowns in the front of the upper jaw did not lead to any noticeable increase in volume of the lacking papillae

Author, year	Outcome	Key takeaway
	Saline • Baseline: 2.3 (1.2) • 3 months: 2.2 (1.2) • 6 months: 2.2 (1.2) Mean (SD) change from the baseline HA • 3 months: –0.23 (0.68) ($p = 0.296$) • 6 months: –0.09 (0.63) ($p = 0.640$) Saline • 3 months: –0.10 (0.39) ($p = 0.443$) • 6 months: –0.15 (0.41) ($p = 0.279$) Mean (SD) black triangle area (mm^2) HA gel • Baseline: 0.51 (0.31) • 3 months: 0.47 (0.20) • 6 months: 0.52 (0.26) Saline • Baseline: 0.51 (0.25) • 3 months: 0.49 (0.23) • 6 months: 0.54 (0.27) Mean (SD) change from the baseline HA gel • 3 months: –0.04 (0.15) ($p = 0.413$) • 6 months: –0.01 (0.10) ($p = 0.734$) Saline • 3 months: –0.02 (0.07) ($p = 0.424$) • 6 months: 0.03 (0.10) ($p = 0.382$) Mean (SD) change from the base in gingival volume (mm^2) HA gel • 3 months: 0.10 (0.23) Saline • 3 months: –0.02 (0.23) Mean (SD) probing depth (mm) HA gel • Baseline: 2.0 (0.5) • 3 months: 2.0 (0.4) • 6 months: 2.1 (0.4) Saline • Baseline: 2.0 (0.6) • 3 months: 2.0 (0.6) • 6 months: 2.1 (0.4) Mean (SD) change from the baseline HA gel • 3 months: –0.05 (0.35) ($p = 0.680$) • 6 months: 0.10 (0.47) ($p = 0.484$) Saline • 3 months: 0.03 (0.63) ($p = 0.902$) • 6 months: 0.06 (0.53) ($p = 0.718$) Mean (SD) CAL (mm) HA gel • Baseline: 2.1 (0.5) • 3 months: 2.0 (0.4) • 6 months: 2.2 (0.4) Saline • Baseline: 2.2 (0.7) • 3 months: 2.2 (0.6) • 6 months: 2.2 (0.4) Mean (SD) change from the baseline HA gel • 3 months: –0.10 (0.34) ($p = 0.341$) • 6 months: 0.05 (0.39) ($p = 0.709$) Saline • 3 months: 0.01 (0.62) ($p = 0.950$) • 6 months: 0.06 (0.52) ($p = 0.941$) Mean (SD) percent of BoP HA gel Baseline: 9.1 (12.6) • 3 months: 25.0 (25.0) • 6 months: 25.0 (25.0) Saline • Baseline: 32.5 (16.9) • 3 months: 45.0 (30.1) • 6 months: 27.5 (18.4)	

Author, year	Outcome	Key takeaway
	Mean (SD) change from the baseline HA gel • 3 months: 15.9 (16.9) ($p = 0.011$) • 6 months: 15.9 (25.7) ($p = 0.067$) Saline • 3 months: 12.5 (24.3) ($p = 0.138$) • 6 months: –5.0 (25.8) (p = 0.555) Mean (SD) percent of plaque HA gel • Baseline: 31.8 (37.2) • 3 months: 38.6 (34.2) • 6 months: 40.9 (35.8) Saline • Baseline: 32.5 (29.0) • 3 months: 42.5 (29.0) • 6 months: 27.5 (24.9) Mean (SD) change from the baseline HA gel • 3 months: 6.8 (38.9) ($p = 0.574$) • 6 months: 9.1 (49.1) ($p = 0.553$) Saline • 3 months: 10.0 (35.8) ($p = 0.399$) • 6 months: –5.0 (36.9) ($p = 0.678$) Mean (SD) radiographic ABH (mm) HA gel • Baseline: 3.6 (0.7) • 6 months: 3.6 (0.7) Saline • Baseline: 3.3 (1.3) • 6 months: 3.1 (1.3) Mean (SD) change from the baseline HA gel • 6 months: 0.06 (0.23) ($p = 0.444$) Saline • 6 months: 0.18 (0.26) (p=0.080) Mean (SD) esthetic appearance measured using VAS[c] – Patient HA gel Baseline: 21.8 (23.8) • 3 months: 11.2 (9.1) • 6 months: 13.7 (11.7) Saline • Baseline: 30.2 (26.6) • 3 months: 17.9 (15.6) • 6 months: 18.1 (13.0) Mean (SD) change from the baseline HA gel • 3 months: –10.6 (22.2) ($p = 0.143$) • 6 months: –8.1 (23.0) ($p = 0.269$) Saline • 3 months: –12.3 (20.8) ($p = 0.094$) • 6 months: –12.1 (20.0) ($p = 0.088$) Mean (SD) esthetic appearance measured using VAS[c] – Examiner HA gel • Baseline: 23.6 (8.4) • 3 months: 20.8 (5.0) • 6 months: 19.9 (6.2) Saline • Baseline: 27.7 (14.9) • 3 months: 25.4 (9.5) • 6 months: 24.8 (10.5) Mean (SD) change from the baseline HA gel • 3 months: –2.7 (6.9) ($p = 0.222$) • 6 months: –3.6 (7.7) ($p = 0.146$) Saline 3 months: –2.3 (6.7) ($p = 0.304$) 6 Months: –2.9 (5.6) ($p = 0.135$)	

Author, year	Outcome	Key takeaway
	Mean (SD) pain intensity measured using VAS HA gel • During injection – 1st injection: 57.1 (23.6) ($p = 0.388$ vs saline) – 2nd injection: 56.2 (19.4) ($p = 0.507$ vs saline) • Within 1 week after injection –1st injection: 27.6 (28.6) ($p = 0.034$ vs saline) –2nd injection: 25.8 (38.3) ($p = 0.064$ vs saline) Saline • During injection – 1st injection: 49.2 (16.3) – 2nd injection: 61.9 (19.3) • Within 1 week after injection – 1st injection: 5.0 (13.8) – 2nd injection: 1.7 (5.4)	
Shawky, 2017[10]	Percentage of sites with PMGI[d] HA gel • Baseline – PMGI score 1: 20.0 (100.0) – PMGI score 2: 0.0 (0.0) – PMGI score 3: 0.0 (0.0) • 3 weeks – PMGI score 1: 20.0 (100.0) – PMGI score 2: 0.0 (0.0) – PMGI score 3: 0.0 (0.0) • 3 months – PMGI score 1: 20.0 (100.0) – PMGI score 2: 0.0 (0.0) – PMGI score 3: 0.0 (0.0) • 6 Months – PMGI score 1: 18.0 (90.0) – PMGI score 2: 2.0 (10.0) – PMGI score 3: 0.0 (0.0) Radiesse gel • Baseline – PMGI score 1: 20.0 (100.0) – PMGI score 2: 0.0 (0.0) – PMGI score 3: 0.0 (0.0) • 3 weeks – PMGI score 1: 17.0 (85.0) – PMGI score 2: 1.0 (5.0) – PMGI score 3: 2.0 (10.0) • 3 months – PMGI score 1: 18.0 (90.0) – PMGI score 2: 2.0 (10.0) – PMGI score 3: 0.0 (0.0) • 6 Months – PMGI score 1: 18 (90.0) – PMGI score 2: 2.0 (10.0) – PMGI score 3: 0.0 (0.0) Percentage of sites with PPI[e] HA gel • Baseline – PPI score 1: 0.0 (0.0) – PPI score 2: 17.0 (85.0) – PPI score 3: 3.0 (15.0) • 3 weeks – PPI score 1: 15.0 (75.0) – PPI score 2: 5.0 (25.0) – PPI score 3: 0.0 (0.0) • 3 months – PPI score 1: 11.0 (55.0) – PPI score 2: 9.0 (45.0) – PPI score 3: 0.0 (0.0)	It was concluded that both fillers were biocompatible and safe for Tx of IDP deficiency. Though, Radiesse gel was more effective and long-lasting compared with HA.

Author, year	Outcome	Key takeaway
	• 6 Months – PPI score 1: 10 (50.0) – PPI score 2: 9.0 (45.0) – PPI score 3: 1.0 (5.0) Radiesse gel • Baseline – PPI score 1: 0.0 (0.0) – PPI score 2: 16.0 (80.0) – PPI score 3: 4.0 (20.0) • 3 weeks – PPI score 1: 18.0 (90.0) – PPI score 2: 2.0 (10.0) – PPI score 3: 0.0 (0.0) • 3 months – PPI score 1: 18 (90.0) – PPI score 2: 2.0 (10.0) – PPI score 3: 0.0 (0.0) • 6 months – PPI score 1: 17.0 (85.0) – PPI score 2: 2.0 (15.0) – PPI score 3: 0.0 (0.0) Probing depth (median, min–max) HA gel • Baseline: 1.0 (1.0–1.3) • 3 weeks: 1.0 (1.0–1.5) • 3 months: 1.0 (1.0–1.5) • 6 months: 1.0 (1.0–1.5) Radiesse gel: • Baseline: 1.0 (1.0–2.0) • 3 weeks: 1.0 (1.0–2.0) • 3 months: 1.0 (1.0–2.0) • 6 months: 1.0 (1.0–2.0) Mean (SD) probing depth HA gel • Baseline: 1.06 (0.12) • 3 weeks: 1.10 (0.21) • 3 months: 1.10 (0.21) • 6 months: 1.10 (0.21) Radiesse gel • Baseline: 1.05 (0.22) ($p = 0.317$) • 3 weeks: 1.08 (0.24) ($p = 0.432$) • 3 months: 1.10 (0.31) ($p = 0.485$) • 6 months: 1.10 (0.31) (p =0.485) Median (min–max) CAL HA gel • Baseline: 0.0 (0.0–2.0) • 3 weeks: 0.0 (0.0–1.5) • 3 months: 0.0 (0.0–1.5) • 6 months: 0.0 (0.0–2.0) Radiesse gel Baseline: 0.0 (0.0–2.0) • 3 weeks: 0.0 (0.0–1.0) • 3 months: 0.0 (0.0–1.0) • 6 months: 0.0 (0.0–1.0) Mean (SD) CAL HA gel • Baseline: 0.20 (0.52) • 3 weeks: 0.13 (0.39) • 3 months: 0.13 (0.39) • 6 months: 0.15 (0.49) Radiesse gel • Baseline: 0.20 (0.52) ($p = 1.000$) • 3 weeks: 0.05 (0.22) ($p = 0.534$) • 3 months: 0.05 (0.22) ($p = 0.534$) • 6 months: 0.05 (0.22) ($p = 0.534$) Median (min–max) PPI[e] HA gel • Baseline: 2.0 (2.0–3.0) • 3 weeks: 1.0 (1.0–2.0) • 3 months: 1.0 (1.0–2.0) • 6 months: 1.5 (1.0–3.0)	

Author, year	Outcome	Key takeaway
	Radiesse gel • Baseline: 2.0 (2.0–3.0) • 3 weeks: 1.0 (1.0–0.0) • 3 months: 1.0 (1.0–2.0) • 6 months: 1.0 (1.0–2.0) Mean (SD) PPI[e] HA gel • Baseline: 2.15 (0.37) • 3 weeks: 1.25 (0.44) • 3 months: 1.45 (0.51) • 6 months: 1.55 (0.60) Radiesse gel • Baseline: 2.20 (0.41) ($p = 0.681$) • 3 weeks: 1.10 (0.31) ($p = 0.218$) • 3 months: 1.10 (0.31) ($p = 0.014$) • 6 months: 1.15 (0.37) ($p = 0.018$) Mean (SD) height of the IDP HA gel • Baseline: 3.81 (0.49) • 3 weeks: 4.31 (0.67) • 3 months: 4.28 (0.64) • 6 months: 4.10 (0.59) Radiesse gel • Baseline: 3.81 (0.49) ($p = 0.897$) • 3 weeks: 5.12 (0.40) ($p < 0.001$) • 3 months: 5.09 (0.43) ($p < 0.001$) • 6 months: 5.10 (0.43) ($p < 0.001$) Median (min–max) % of change of IDP height HA gel • 3 weeks: 8.6 (0.0–36.5) • 3 months: 4.5 (0.0–38.1) • 6 months: 2.2 (–0.7–37.8) Radiesse gel • 3 weeks: 30.3 (0.2–82.6) • 3 months: 28.9 (0.2–83.0) • 6 months: 28.9 (0.2–83.0) Mean (SD) % of change of IDP height HA gel • 3 weeks: 13.3 (13.4) • 3 months: 12.9 (13.8) • 6 months: 7.9 (12.5) Radiesse gel • 3 weeks: 35.3 (18.6) ($p = 0.001$) • 3 months: 34.5 (18.6) ($p = 0.001$) • 6 months: 34.8 (18.6) ($p = 0.001$)	
Abdelraouf, 2019[11]	Mean (SD) change from the baseline in black triangle height (mm) HA gel • 0–3 months: –0.31 (0.25) ($p = 0.025$) • 3–6 months: –0.06 (0.17) ($p = 0.822$) • 0–6 months: –0.25 (0.26) ($p = 0.047$) Saline • 0–3 months: –0.07 (0.18) • 3–6 months: 0.04 (0.13) • 0–6 months: –0.03 (0.13) Mean (SD) % change from baseline in black triangle area HA gel • 0–3 months: –36.5 (24.4) ($p < 0.001$) • 3–6 months: –11.8 (30.3) ($p = 0.224$) • 0–6 months: –45.0 (28.5) ($p < 0.001$) Saline • 0–3 months: –0.9 (10.6) • 3–6 months: 0.9 (8.9) • 0–6 months: –2.0 (11.4) Mean (SD) satisfaction score HA gel • 6 months: 45.0 (12.65) ($p = 0.002$) Saline • 6 months: 27.86 (12.65)	HA gel was successfully used to reconstruct interdental papillary deficiencies, and patient satisfaction levels were encouraging.

Author, year	Outcome	Key takeaway
Mandel, 2020[12]	Mean (SD) black triangle area (%) (pixel count) Revident (test) • Baseline: 100.0 (0.0) • Immediately after Tx: 81.0 (16.38) • 1 week: 84.8 (18.9) • 1 month: 86.1 (22.6) Revident (untreated control) • Baseline: 100.0 (0.0) • Immediately after Tx: 100 (0.0) • 1 week: 99.3 (3.6) • 1 month: 100.4 (4.6) Flex barrier (test) • Baseline: 100 (0.0) • Immediately after Tx: 83.8 (17.1) • 1 week: 91.2 (13.24) • 1 month: 96.0 (8.8) Flex barrier (untreated control) • Baseline: 100.0 (0.0) • Immediately after Tx: 100 (0) • 1 week: 100.0 (1.1) • 1 month: 100.1 (0.9)	This study showed that both Flex Barrier and Revident have clinical applicability. However, Revident showed longer-lasting improvements compared with Flex Barrier. Additional trials are necessary to enhance the effectiveness of multiple-application protocols for the treatment of gingival black triangles.
Kapoor, 2022[13]	Mean (SD) black triangle area (units not reported) HA gel • Baseline: 1.58 (0.46) • 3 weeks: 1.20 (0.37) • 3 months: 0.98 (0.15) • 6 months: 0.42 (0.26) Placebo • Baseline: 1.54 (0.54) • 3 weeks: 1.86 (0.62) • 3 months: 1.93 (0.55) • 6 months: 1.85 (0.52) Mean (SD) black triangle width (units not reported) HA gel • Baseline: 2.81 (1.14) • 3 weeks: 2.10 (1.06) • 3 months: 1.21 (0.64) • 6 months: 0.55 (0.46) Placebo • Baseline: 2.96 (1.00) • 3 weeks: 3.17 (0.97) • 3 months: 2.96 (0.99) • 6 months: 2.93 (0.97) Mean (SD) black triangle height (units not reported) HA gel • Baseline: 3.86 (2.01) • 3 weeks: 2.80 (1.59) • 3 months: 1.32 (0.76) • 6 months: 0.58 (0.40) Placebo • Baseline: 5.02 (1.84) • 3 weeks: 5.31 (1.44) • 3 months: 4.97 (1.45) • 6 months: 4.95 (1.34) Percentage of sites with no clinical signs of black triangle • HA gel: 63.3% • Placebo: 0.0% Percentage of sites with clinically observable black triangle (%) • HA gel: 36.7% • Placebo: 13.3% Percentage of sites with no change (%) • HA gel: 0.0% • Placebo: 90%	HA is an alternative noninvasive volumizing therapy for interdental papilla deficiency. Because it does not result in an unfavorable response, it is biocompatible. Gingival fibroblasts' proliferative and migrating capacities are significantly impacted by HA gel.
Bal, 2023[14]	Mean (SD) black triangle width (mm) HA • Baseline (n = 18): 0.94 (0.35) • 3 weeks (n = 18): 0.81 (0.29) • 6 weeks (n = 18): 0.68 (0.32) • 12 weeks (n = 17): 0.60 (0.36) HA + PRGF • Baseline (n = 18): 0.77 (0.24) • 3 weeks (n = 18): 0.69 (0.21) • 6 weeks (n = 18): 0.64 (0.21) • 12 weeks (n = 17): 0.54 (0.19) Mean (SD) change from the baseline HA • 3 weeks (n = 18): 0.14 (0.22) (p = 0.019) • 6 weeks (n = 18): 0.26 (0.30) (p = 0.002) • 12 weeks (n = 17): 0.34 (0.36) (p = 0.001) HA + PRGF • 3 weeks (n = 18): 0.08 (0.1) (p = 0.003) • 6 weeks (n = 18): 0.13 (0.12) (p < 0.001) • 12 weeks (n = 17): 0.23 (0.17) (p < 0.001) Mean (SE) Tx difference (HA vs. HA + PRGF) • Baseline: 0.18 (0.1) (p = 0.08) • 3 weeks: 0.12 (0.08) (p = 0.16) • 6 weeks: 0.04 (0.09) (p = 0.63) • 12 weeks: 0.05 (0.1) (p = 0.59) Mean (SD) black triangle height (mm) HA • Baseline (n = 18): 1.28 (0.69) • 3 weeks (n = 18): 1.18 (0.68) • 6 weeks (n = 18): 1.04 (0.53) • 12 weeks (n = 17): 0.89 (0.61) HA + PRGF • Baseline (n = 18): 1.12 (0.32) • 3 Weeks (n = 18): 0.90 (0.11) • 6 Weeks (n = 18): 0.56 (0.20) • 12 Weeks (n = 17): 0.33 (0.21) Mean (SD) change from the baseline HA • 3 weeks (n = 18): 0.10 (0.06) (p < 0.001) • 6 weeks (n = 18): 0.24 (0.17) (p < 0.001) • 12 weeks (n = 17): 0.41 (0.23) (p < 0.001) HA + PRGF • 3 weeks (n = 18): 0.22 (0.3) (p < 0.007) • 6 weeks (n = 18): 0.56 (0.3) (p < 0.001) • 12 weeks (n = 17): 0.80 (0.3) (p < 0.001) Mean (SE) Tx difference (HA vs HA + PRGF) • Baseline: 0.16 (0.18) (p = 0.37) • 3 weeks: 0.28 (0.16) (p = 0.09) • 6 weeks: 0.49 (0.13) (p = 0.001) • 12 weeks: 0.56 (0.16) (p = 0.001) Mean (SD) black triangle area (mm^2) HA • Baseline (n = 18): 0.61 (0.45) • 3 weeks (n = 18): 0.54 (0.49) • 6 weeks (n = 18): 0.42 (0.41) • 12 weeks (n = 17): 0.36 (0.44) HA + PRGF • Baseline (n = 18): 0.42 (0.16) • 3 weeks (n = 18): 0.31 (0.11) • 6 weeks (n = 18): 0.17 (0.06) • 12 weeks (n = 17): 0.09 (0.05) Mean (SD) change from the baseline HA • 3 weeks (n = 18): 0.07 (0.13) (p = 0.028) • 6 weeks (n = 18): 0.19 (0.15) (p < 0.001) • 12 weeks (n = 17): 0.25 (0.19) (p < 0.001) HA + PRGF • 3 weeks (n = 18): 0.11 (0.12) (p = 0.001) • 6 weeks (n = 18): 0.25 (0.14) (p < 0.001) • 12 weeks (n = 17): 0.34 (0.16) (p < 0.001) Mean (SD) percent change from baseline HA • 3 weeks (n = 18): 12.07 (8.96) • 6 weeks (n = 18): 20.24 (10.8) • 12 weeks (n = 17): 57.62 (21.78)	While HA gel has been shown to be a promising injectable treatment for interdental papillary deficiency, PRGF may also be utilized in conjunction with HA to provide a notable adjuvant effect. More clinical research with longer follow-up times, larger sample sizes, and tooth shape standardization is needed to fully understand the adjuvant effect of PRGF in combination with HA.

Author, year	Outcome	Key takeaway
	HA + PRGF • 3 weeks (n = 18): 15.94 (14.41) (p = 0.340) • 6 weeks (n = 18): 49.30 (18.01) (p = 0.000) • 12 weeks (n = 17): 77.42 (16.7) (p = 0.006) Mean (SE) Tx difference (HA vs. HA + PRGF) • Baseline: 0.19 (0.11) (p = 0.11) • 3 weeks: 0.22 (0.12) (p = 0.07) • 6 weeks: 0.24 (0.10) (p = 0.019) • 12 weeks: 0.27 (0.11) (p = 0.017) Mean (SD) deficient volume (mm^3) HA • Baseline (n = 18): 1.79 (1.32) • 3 weeks (n = 18): 1.20 (1.15) • 6 weeks (n = 18): 0.71 (0.67) • 12 weeks (n = 17): 0.46 (0.61) HA + PRGF • Baseline (n = 18): 1.42 (0.62) • 3 weeks (n = 18): 0.80 (0.31) • 6 weeks (n=18): 0.35 (0.15) • 12 weeks (n = 17): 0.12 (0.07) Mean (SD) change from the baseline HA • 3 weeks (n = 18): 0.59 (0.49) (p<0.001) • 6 weeks (n = 18): 1.08 (0.74) (p<0.001) • 12 weeks (n = 17): 1.33 (0.86) (p<0.001) HA + PRGF • 3 weeks (n = 18): 0.63 (0.39) (p < 0.001) • 6 weeks (n = 18): 1.07 (0.55) (p = 0.000) • 12 weeks (n = 17): 1.33 (0.60) (p = 0.000) Mean (SD) percent change from baseline HA • 3 weeks (n = 18): 23.60 (15.87) • 6 weeks (n = 18): 56.03 (20.55) • 12 weeks (n = 17): 81.42 (17.20) HA + PRGF • 3 weeks (n = 18): 42.28 (11.91) (p = 0.000) • 6 weeks (n = 18): 73.14 (13.83) (p = 0.006) • 12 weeks (n = 17): 91.19 (5.70) (p = 0.033) Mean (SE) Tx difference (HA vs. HA + PRGF) • Baseline: 0.37 (0.34) (p = 0.29) • 3 weeks: 0.41 (0.28) (p = 0.16) • 6 weeks: 0.36 (0.16) (p = 0.032) • 12 weeks: 0.33 (0.15) (p = 0.032)	

ABH – alveolar bone height; BoP – bleeding on probing; CAL – clinical attachment level; HA – hyaluronic acid; IDP – interdental papilla; iPRF – injectable platelet-rich fibrin; MPIS – modified papilla index score; mm – millimeter; PMGI – papillary marginal gingival index; PPI – papilla presence index; PT–CP – distance between the papilla tip and the contact point; SD – standard deviation; SE – standard error; Tx – treatment.

[a] Quantitative analysis of the IDP was performed using the MPIS as follows:
• 0 – no papilla is present;
• 1 – less than half of the papilla height is present;
• 2 – half or more of the papilla height is present, but the papilla does not extend all the way to the contact point of the teeth;
• 3 – the papilla completely fills the proximal space;
• 4 – the papilla is hyperplastic, and excess tissue is present.

[b] PT–CP refers to the distance, in mm, between the papilla tip and the contact point.

[c] VAS is a validated one-dimensional scale that measures pain intensity, rated from 0 to 100 mm:
• 0–4 mm – no pain;
• 5–44 mm – mild pain;
• 45–74 mm – moderate pain;
• 75–100 mm – severe pain.

[d] PMGI is used to score gingival inflammation on a scale from 0 to 3:
• 0 – no inflammation;
• 1 – mild inflammation;
• 2 – moderate inflammation;
• 3 – severe inflammation.

[e] PPI is a newer classification of interpapillary height and is scored from 1 to 4:
• 1 – the papilla is completely present;
• 2 – the papilla is no longer completely present, but the interdental cementoenamel junction is not visible;
• 3 – the papilla is no longer completely present, and the interdental cementoenamel junction is visible;
• 4 – the papilla is no longer completely present, and both the buccal and interdental cementoenamel junctions are visible.

by Ni et al. found no significant differences between HA gel and saline solution in terms of black triangle area and gingival papilla height.[1] However, the results of in vitro analyses revealed a significantly increased rate of proliferation and migration of human gingival fibroblasts (HGF) following HA treatment, with the effect being more pronounced at higher concentrations.[1]

In the three studies that reported patient-reported satisfaction, a visual analog scale (VAS) was used to assess dental esthetics before and after HA treatment. All 3 studies reported significantly higher satisfaction with esthetic appearance following HA treatment.

Quality assessment of the included studies

One study had a low risk of bias across all the 7 domains, and 2 studies reported a low risk in 6 domains and an unclear risk of bias in 1 domain. Of the remaining studies (n = 6), 5 reported unclear risk of bias in 3 of the 7 domains and 1 study reported a high risk of bias in 1 domain (i.e., blinding of participants and personnel) (Table 4).

Overall, this review provides a descriptive summary of the included studies. The considerable heterogeneity across the included studies precluded the possibility of performing a meta-analysis.

Discussion

This systematic review provides the most contemporary evidence on the efficacy, safety and HRQoL outcomes of HA treatment in patients with IDP deficiency. Evidence from this review indicates that HA, whether used alone or in combination with other therapies (i.e., PRGF, injectable carboxymethyl chitosan hydrogel or iPRF), represents an effective and minimally invasive option for the treatment of IDP deficiency. It is important to highlight that none of the included RCTs provided data on the safety of HA treatment or its effect on HRQoL in this population.

The use of HA has garnered considerable interest for its potential role in restoring unesthetic defects in the papilla. Data from RCTs have shown favorable therapeutic benefits of HA in treating IDP deficiency.[1,3,5,8,10–14] This review specifically aimed to synthesize the existing evidence on the efficacy, safety and HRQoL outcomes of HA treatment, and to provide meaningful insights into the use of HA treatment for patients with IDP defects. Across the 9 RCTs included in this literature review, HA was used either alone (n = 6)[1,9–13] or in combination with other therapies (n = 3) (i.e., PRGF, injectable carboxymethyl chitosan hydrogel or iPRF).[5,8,14] Although there was considerable heterogeneity across the included studies in terms of population, sample size and treatment duration, HA was found to confer positive benefits as compared to HA alone or control arms. Consistent with previously published reviews,[3,6,7,15,16] HA was reported to

Table 4. Quality assessment of the included randomized controlled trials (RCTs) ($N = 9$), using the Cochrane risk-of-bias assessment tool (RoB)[18]

Author, year	Random sequence generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias, ideally prespecified
Ni, 2021[1]	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk
Fakher, 2023[5]	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Abdelkader, 2022[8]	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Bertl, 2016[9]	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk
Shawky, 2017[10]	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Abdelraouf, 2019[11]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Mandel, 2020[12]	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Kapoor, 2022[13]	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk
Bal, 2023[14]	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk

exert a positive effect on an array of clinical outcomes, including black triangle dimensions (i.e., area, height and width).[1,5,8,11–14] Additionally, patients were found to be adequately satisfied with their esthetic appearance after HA treatment.[5,8,11] This finding is of significant importance, as satisfaction with treatment is important for promoting acceptance of any intervention and improving health outcomes among patients with IDP deficiency.

The results of this review are consistent with those of previously published studies[3,6,7,15,16] and closely resemble the findings of a recent systematic review,[6] which identified HA injections as an effective and minimally invasive method for reconstructing a lost IDP. The key difference is that our review extends beyond clinical outcomes alone. We emphasize that, despite numerous clinical trials, the safety of HA and its effects on HRQoL in patients with IDP deficiency remain poorly investigated.

This review has some inherent limitations. The small sample sizes and limited number of RCTs included in this review are the major limitations. This highlights the need for robust, well-designed trials in this patient population. Another limitation of the review is the heterogeneity observed with regard to study design, treatment protocols and outcome measures, which prevents us from drawing robust conclusions on the efficacy and safety of HA in the treatment of IDP deficiency. As this review included results from RCTs only, its findings cannot be generalized to non-RCT interventions or real-world dental practice. Patients in the included studies had only a short-term exposure to HA, indicating a need for further research with longer treatment durations to better understand the long-term benefits of HA treatment. Most of the included RCTs in this review were judged to have an unclear risk of bias in some domains, which might have affected the study findings. Only studies published in English were included; thus, there is a high risk of missing important studies published in other languages. Finally, few studies reported data on safety outcomes, and no HRQoL data were reported across the included studies.

Despite these limitations, several strengths of the current systematic review merit consideration. First, this review was conducted in accordance with the PRISMA guidelines, which ensure the use of structured and optimal methodology to synthesize the evidence. The employed search methodology was extensive and rigorous, incorporating data from both published and unpublished studies, as well as manual and bibliographic search to ensure comprehensive coverage. This approach aimed to include studies from diverse populations and geographic regions, minimizing the risk of excluding any demographic groups. Finally, the studies included in this review were critically appraised using the Cochrane risk-of-bias tool, thereby adding further rigor to the review.

Conclusions

Based on the limited published evidence, this systematic review suggests that HA, either alone or in combination with other therapies, may be an effective and minimally invasive treatment option for the restoration of IDP deficiency. However, data on the safety of HA and its impact on HRQoL for IDP-deficient patients are lacking, highlighting the need for future clinical studies to address these aspects.

Ethics approval and consent to participate

Not applicable.

Data availability

The data presented within this manuscript have been extracted from 9 included studies. The data were readily available within the cited articles. The search strategy used for retrieving the studies for the current systematic review are openly available in https://figshare.com at doi:10.6084/m9.figshare.26863660.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Review

Clinical outcomes and efficacy of piezosurgery compared to conventional surgery for cyst enucleation: A systematic review

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Abstract

Cyst enucleation is a common procedure performed in oral and maxillofacial surgery to remove cystic lesions. Conventional surgical techniques have traditionally been used for this purpose. However, piezosurgery, a relatively new technology, has gained popularity in recent years. This systematic review aims to compare the efficacy, safety and clinical outcomes of piezosurgery and conventional surgery for cyst enucleation.

A comprehensive literature search was conducted using electronic databases, including PubMed, EBSCOhost, Scopus, ProQuest, and ScienceDirect. Studies published from inception up to May 2023 were included. Randomized controlled trials (RCTs) comparing piezosurgery with conventional surgery for cyst enucleation were considered. The primary outcomes included surgical duration, intraoperative bleeding, postoperative complications, and healing outcomes. Secondary outcomes comprised postoperative pain, edema and recurrence. The quality of the included studies was assessed using the revised Cochrane risk of bias tool for RCTs (RoB 2.0).

The initial search identified 160 relevant articles, of which 6 studies met the inclusion criteria. Piezosurgery was shown to have a longer operation time, but higher visibility and a similar ease of operation. Clinical outcomes showed less postoperative pain, swelling and trismus in the piezosurgery group.

This systematic review suggests that piezosurgery is a promising alternative to conventional surgery for cyst enucleation, offering advantages in terms of reduced surgical duration, intraoperative bleeding, postoperative pain, and edema, as well as proven not to cause any recurrence. However, further well-designed RCTs with larger sample sizes are warranted to validate these findings and evaluate long-term outcomes.

Keywords: piezosurgery, enucleation, odontogenic cyst, conventional surgery

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Highlights

- Piezosurgery provides a more conservative approach for odontogenic cyst enucleation by enabling precise bone cutting while preserving the adjacent anatomical structures.
- Compared with conventional rotary instruments, piezosurgery reduces postoperative complications, including pain, swelling, and the risk of soft tissue and neurovascular injury.
- Piezosurgery improves surgical visibility and reduces intraoperative bleeding through the cavitation effect, facilitating safer cyst removal near vital structures.
- Despite longer operative times and technical limitations, piezosurgery demonstrates favorable clinical outcomes and potential advantages in the management of odontogenic cysts.

Introduction

Odontogenic cysts are common benign lesions that can arise from the epithelial remnants of tooth development or the inflammation of the dental pulp. They occur in 2.5% of the general population. These cysts can grow in size and cause bony expansion, the destruction of the surrounding bone, and the displacement of the adjacent teeth. They are usually asymptomatic and detected incidentally on routine radiographs. However, some cysts may cause pain, swelling or other complications.[1]

The treatment of odontogenic cysts varies depending on several factors; however, surgical intervention remains the mainstay of management. In recent years, more conservative surgical approaches have gained increasing support, as they are considered effective in treating odontogenic cysts while preserving the surrounding bone and teeth. Consequently, surgical treatment is increasingly designed to be as conservative as possible.[2–4]

Apart from the operative approach, more conservative techniques have also been developed for the treatment of odontogenic cysts. Piezosurgery is a specialized technique that uses non-heat-generating ultrasonic vibrations to cut and shape bone instead of conventional rotary instruments, which are more invasive and generate heat. It is thought to provide greater control and precision, and is associated with reduced trauma to the surgical site. Its use in oral and maxillofacial surgery has been reported to result in better clinical outcomes and fewer complications as compared to conventional techniques. However, little is known about its use in the surgical management of odontogenic cysts.[2–4]

The purpose of this systematic review was to compare the clinical outcomes of odontogenic cyst removal with piezosurgery and conventional surgery.

Methods

Protocol and registration

This literature review was conducted in accordance with the *Cochrane Handbook for Systematic Reviews of Interventions*, and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

Eligibility criteria

Studies were screened based on the inclusion criteria of the population, intervention, comparison, outcome, and study design (PICOS) strategy. Articles focusing on other outcomes unrelated to piezosurgery for cyst removal, editorials, reviews, magazines, letters, and foreign articles were excluded.

Patients of any age, with good general health or controlled systemic disease, diagnosed with odontogenic cysts and indicated for jawbone cyst removal were included as the study population (P). The intervention investigated was piezosurgery (I), which was compared with conventional enucleation, the gold standard conservative treatment for odontogenic cysts (C). The primary outcomes (O) included the incidence of complications (e.g., hemorrhage, soft tissue injury, pain, swelling, and trismus), the duration of surgery, reduction in bone density, the ease of operation, and surgical visibility. Randomized clinical trials (RCTs), with or without blinding, as well as case series and case reports involving more than 5 cases, were eligible for inclusion (S).

Information sources

A comprehensive literature search was conducted in 5 electronic databases: PubMed; EBSCOhost; Scopus; ProQuest; and ScienceDirect. No date restrictions were applied, and all relevant studies published in English from database inception up to May 2023 were considered. In addition, the reference lists of the included studies were hand-searched to identify potentially eligible articles not captured by the electronic search.

Search strategy

The search strategy combined the keywords “enucleation” OR “cyst removal”, “piezosurgery” OR “piezoelectric”, and “odontogenic cyst” OR “jaw cyst”, together with relevant synonyms. The search terms and their

combinations were adapted to the advanced search requirements of each database.

Data extraction

Data were extracted after the full texts of the included studies had been reviewed by the authors. The extracted data included: (1) first author and publication year; (2) study design; (3) sample size; (4) population characteristics; (5) cyst location; (6) treatment outcomes; (7) follow-up period; and (8) recurrence.

Quality assessment

The risk of bias was assessed using the revised Cochrane risk of bias tool for RCTs (RoB 2.0). The domains assessed included selection bias, performance bias, attrition bias, detection bias, and reporting bias. Three reviewers (UPG, LAL and SP) independently assessed the included studies. Each study was rated as having a low risk of bias, some concerns, or a high risk of bias according to the RoB 2.0 guidance. Studies in which all domains were rated as low risk were assigned an overall judgment of low risk of bias. Studies with at least one domain rated as having some concerns, but no domains rated as high risk, were judged to have some concerns. Studies with at least one domain rated as high risk were judged to have an overall high risk of bias.

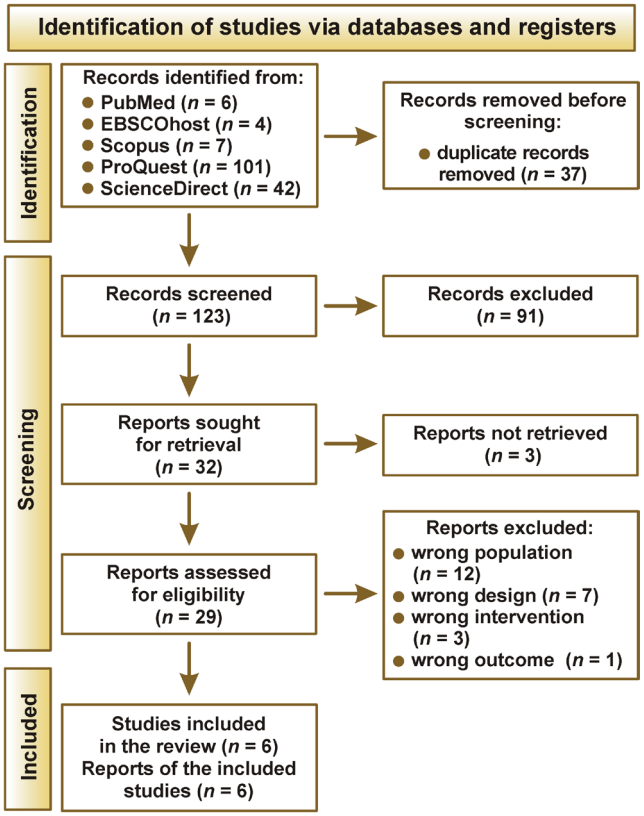


Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of the study

Results

Study selection

The search of 5 electronic databases yielded 160 records, of which 37 were duplicates. After duplicate removal, 123 records remained for title and abstract screening, and 91 were excluded. The remaining 32 reports were sought for full-text retrieval. Following full-text assessment, 29 articles were evaluated against the inclusion and exclusion criteria, and six studies were included in the review. The study selection process is presented in Fig. 1.

Risk of bias

The risk of bias assessment showed that 3 studies had a low risk of bias, while the remaining 3 showed some concerns. A summary of the risk of bias assessment is presented in Fig. 2.

Characteristics of the included studies

The characteristics of the 6 RCTs included in the review[1–6] are presented in Table 1. The dataset consists of the first author, year of publication, study design, population characteristics, cyst location, follow-up period, and clinical outcomes from both the intervention and comparison groups.

Outcomes

Intraoperative outcomes

Several intraoperative outcomes were identified from the included studies, including manipulation complexity, the ease of operation, visibility, and operation duration.

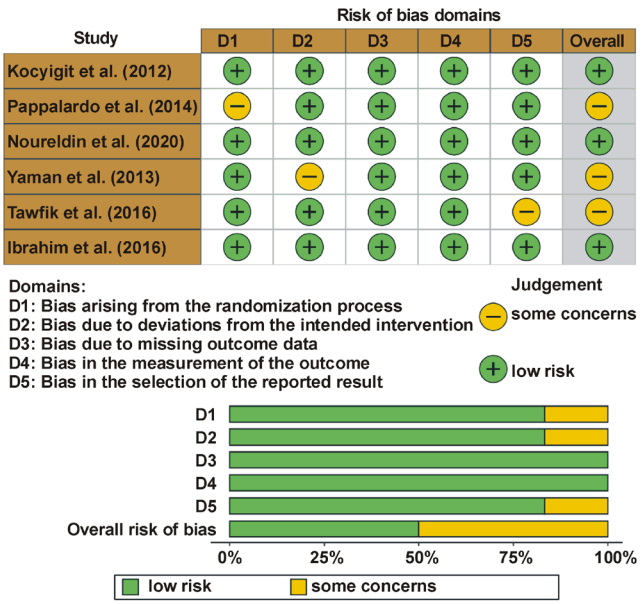


Fig. 2. Risk of bias assessment

Table 1. Characteristics of the studies included in the review

First author	Study design	Population characteristics	Diagnosis	Location	Follow-up period	Clinical outcome	Results	
							conventional surgery	piezosurgery
Kocyigit[1] 2012	RCT	sample size (*n*) (test vs. control) 19 vs. 10 sex (F:M) 13:16 mean age [years] 29.3	radicular cyst	18 maxilla 11 mandible	5–24 months	1. none 2. hemorrhage 3. soft tissue damage 4. manipulation complexity 5. major perforation areas 6. operation duration 7. recurrence	0 5 0 5 5 on time 2	20 0 0 0 0 exceeded 0
Pappalardo[2] 2014	RCT	sample size (*n*) (test vs. control) 40 vs. 40 sex (F:M) 45:35 mean age [years] 42.3	NA	80 mandible	7 days	pain (VAS) swelling (facial edema)	5.06 ±1.37 40.50 ±6.50	1.65 ±0.42* 15.00 ±5.92*
Noureldin[3] 2020	RCT	sample size (*n*) (test vs. control) 7 vs. 7 sex (F:M) 8:6 mean age [years] 40.7	radicular cyst	9 maxilla 5 mandible	1 day 2 days 7 days 3 months	pain (VAS) day 1–2–7 operation duration ease of operation visibility neurosensory deficiency bone defect reduction	8.01–6.71–1.62 44.43 ±12.74 73.57 ±5.56 79.29 ±3.45 2 33.04%	5.95–3.81–1.28 68.00 ±12.45* 77.86 ±4.80 88.57 ±3.78* 0 46.58%*
Yaman[4] 2013	RCT	sample size (*n*) (test vs. control) 34 vs. 34 sex (F:M) 25:43 mean age [years] 35.3	radicular cyst dentigerous cyst residual cyst keratocyst	51 maxilla 31 mandible	18–57 months	complications visibility ease of operation operation duration	– 79.8 ±9.4 80.3 ±7.2 36.0 ±15.3	– 89.4 ±5.2* 75.1 ±8.7 51.0 ±27.9*
Tawfik[5] 2016	RCT	sample size (*n*) (test vs. control) 10 vs. 10 sex (F:M) NA mean age [years] NA	radicular cyst	NA	1–7 days 6 months 12 months	pain swelling trismus (2 days) operation duration bone density (6 months)	4 mild/6 moderate 35.08 ±1.06 4.05 ±0.13 89.10 ±10.92 75.50 ±6.39	6 mild/2 moderate 32.65 ±1.55* 3.80 ±0.18* 104.10 ±13.43* 84.00 ±7.74*
Ibrahim[6] 2016	RCT	sample size (*n*) (test vs. control) 7 vs. 7 sex (F:M) 8:6 mean age [years] 32.2	periapical cyst	14 mandible	1 day 2 days 7 days 1 month 3 months 6 months	soft tissue damage manipulation complexity pain infection tissue dehiscence surface area (6 months) bone density	0 0 7.71 ±0.95 0 3 32.68 ±11.92 666.10 ±193.88	0 0 3.43 ±0.98 0 0 17.39 ±3.44 1,091.34 ±193.96

RCT – randomized controlled trial; F – female; M – male; VAS – visual analog scale; NA – data not available; * statistically significant difference.

Manipulation complexity was reported in 2 studies – by Kocyigit et al.[1] and Ibrahim et al.[6] In the study by Kocyigit et al., manipulation complexity was observed in 5 patients in the conventional group and none in the piezosurgery group.[1] In contrast, Ibrahim et al. reported no cases of manipulation complexity in either group.[6]

The ease of operation, visibility and operation duration were reported in 4 studies included in the analysis.[1,3–5] Kocyigit et al. reported that the piezosurgery group exceeded the expected operation time on average.[1] In comparison with conventional surgery, enucleation using piezosurgery required a longer operation time, as reported by Noureldin and Melek (68.00 ±12.45 min vs. 44.43 ±12.74 min),[3] Yaman and Suer (51.0 ±27.9 min vs. 36.0 ± 15.3 min)[4] and Tawfik et al. (104.10 ±13.43 min vs. 89.10 ±10.92 min)[5]; statistical significance was reported in all 3 studies. The subjective ease of operation was similar between groups in the study by Noureldin and Melek, with no statistically significant difference observed.[3] In contrast, visibility was significantly better with piezosurgery as compared to the conventional group, as reported by Yaman and Suer (89.4 ±5.2 vs. 79.8 ±9.4).[5]

Postoperative outcomes

Sequelae and complications were reported in all 6 included RCTs. The reported complications comprised hemorrhage, soft tissue damage, major perforation area, recurrence, neurosensory deficits, tissue dehiscence, and infection. The reported sequelae included pain, swelling and trismus, while some studies also evaluated changes in bone density.

Bone density changes were reported in 3 included studies – by Noureldin and Melek,[3] Tawfik et al.[5] and Ibrahim et al.[6] In the study by Noureldin and Melek, bone defect reduction was significantly greater in the piezosurgery group as compared to the conventional group (46.58% vs. 33.04%).[3] Similarly, higher bone density was observed in the piezosurgery group than in the control group in the studies by Tawfik et al. (84.00 ±7.74 vs. 75.50 ±6.39)[5] and Ibrahim et al. (1,091.34 ±193.96 vs. 666.10 ±193.88).[6]

Pain, swelling and trismus were reported in 4 studies.[2,3,5,6] Pain was consistently lower in the piezosurgery group as compared to the control group, as reported by Pappalardo and Guarnieri (1.65 ±0.42 vs. 5.06 ±1.37),[2] Noureldin and Melek (5.95 vs. 8.01; 3.81 vs. 6.71; 1.28 vs. 1.62)[3] and Ibrahim et al. (3.43 ±0.98 vs. 7.71 ±0.95).[6] Tawfik et al. also reported milder pain in the piezosurgery group than in the control group.[5] Swelling was also reported to be lower in the piezosurgery group by Pappalardo and Guarnieri (15.00 ±5.92 vs. 40.50 ±6.50)[2] and Tawfik et al. (32.65 ±1.55 vs. 35.08 ±1.06),[5] with statistically significant differences. In addition, trismus was significantly lower in the piezosurgery group as compared to the control group (3.80 ±0.18 vs. 4.05 ± 0.13, as reported by Tawfik et al.[5]

Some complications were reported as having no occurrence in either group in some studies. Ibrahim et al. reported no cases of soft tissue damage or infection in either group,[6] while Kocyigit et al. also reported no occurrence of soft tissue damage in either group.[1]

Discussion

Radicular cysts, the most common odontogenic cysts of the oral cavity, can be managed using various treatment modalities, including enucleation, marsupialization, decompression, and curettage.[5,7] Enucleation followed by the reconstruction of the bony defect is generally the preferred approach, although the spontaneous healing of the enucleated cyst cavity without reconstruction has also demonstrated favorable outcomes.[8] In inflammatory odontogenic cysts, the incomplete removal of the cystic epithelium may increase the risk of recurrence. In addition, adjunctive procedures, such as apicoectomy or tooth extraction, performed during enucleation may adversely affect the prognosis.[7,8]

Surgical enucleation aims to achieve the complete removal of the cystic epithelium, leaving no remnants around the interdental septa or the adjacent teeth. In recent years, piezosurgery, a technique that utilizes piezoelectric technology in oral and maxillofacial surgery, has gained popularity as an alternative to conventional surgical instruments.[9] Introduced by Aro et al. approximately a century after Pierre Curie described the piezoelectric effect in 1880, this technology addresses several limitations of conventional instruments, and its application in bone surgery has become increasingly widespread over the past decade.[10]

The use of traditional hand instruments and surgical burs during cyst enucleation may have several drawbacks, potentially leading to intraoperative complications and an increased risk of recurrence.[11] Rotary instruments, which are commonly used for this procedure, are considered more traumatic than piezosurgical devices. The high- and low-speed rotational movements used during bone removal may contribute to intraoperative and postoperative hemorrhage, which can obstruct the surgical field and make the complete removal of the cystic epithelium and residual tissue more challenging. In contrast, piezosurgery offers the advantage of reducing hemorrhage through the cavitation effect generated by ultrasonic micro-vibrations. This effect helps maintain a clear surgical field, facilitating the identification and removal of cystic remnants during the procedure.[11,12]

Unlike conventional micro-saws, in which blood accumulation within the cutting area can impair visibility, piezosurgery maintains a nearly blood-free operative field during bone cutting procedures. This is attributed to the cavitation effect produced by the irrigation fluid and the ultrasonic vibrations generated by the instrument. The cavitation effect continuously clears blood from the

surgical site, thereby improving visibility. Consequently, bleeding from the surrounding soft tissues is reduced, resulting in lower overall blood loss.[13,14] Furthermore, unlike conventional bone drilling or sawing, piezosurgery enables precise bone cutting while minimizing the risk of injury to the adjacent soft tissues, as it removes mineralized tissue through ultrasonic micro-vibrations rather than rotational or torsional forces.[13]

Another advantage of piezosurgery is its ability to provide continuous irrigation, thereby preventing bone overheating through the integrated saline coolant spray. This is achieved by applying light pressure to the handpiece while maintaining a steady flow of the cooling solution. The combination of continuous irrigation and effective temperature control not only improves the visibility of the surgical field, but also helps protect the bone from thermal injury.[14]

Compared with conventional rotary instruments or drills, piezosurgery requires only minimal pressure from the surgeon, which results in more precise bone cutting. Excessive pressure may restrict the movement of the instrument tip and generate excessive heat. When excessive pressure causes the tip to stop vibrating, indicating heat generation, the device emits a warning tone to alert the surgeon to discontinue cutting and prevent thermal injury to the bone. Piezosurgery is primarily designed for bone cutting while preserving the adjacent soft tissues, although modified settings have also been used for the excision of soft tissue lesions.[15]

Another advantage of piezosurgery is the availability of specialized tips, including straight and angulated designs, which enable precise manipulation, particularly in interdental areas. These tips facilitate access to the surgical site and may reduce the risk of major perforation of the cystic epithelium during enucleation. In contrast, conventional rotary instruments carry a greater risk of injury to the adjacent anatomical structures, such as nerves, the maxillary sinus and the nasal mucosa, particularly when used in anatomically challenging areas. Piezosurgery enhances surgical safety by selectively cutting mineralized tissue while minimizing injury to the surrounding soft tissues.[11]

For example, one study reported successful enucleation of a cyst located adjacent to the mental nerve using piezosurgery, with no temporary or permanent paresthesia observed.[16] This finding highlights the potential safety advantages of piezosurgery when operating in close proximity to critical anatomical structures.

The impact of mechanical instruments on bone structure and cell viability is an important consideration in surgery, as even brief exposure to elevated temperatures may result in cellular damage and tissue necrosis. Previous studies have shown that bone harvested using conventional instruments, such as round burs, spiral implant burs or bone scrapers, may be unsuitable for grafting because of the absence of viable osteocytes and the predominance of nonviable bone tissue. In contrast, studies evaluating piezoelectric surgery have demonstrated improved bone cell viability, supporting earlier findings on the favorable effects of piezoelectric devices on bone chip morphology and cell survival during bone harvesting.[14]

Notably, Stübinger et al. demonstrated that autologous bone harvested from the zygomaticomaxillary region using a piezoelectric device could be successfully used for bone augmentation and the esthetic placement of oral implants following a 5-month healing period.[7] Similarly, Happe reported that bone grafts harvested from the mandibular ramus using piezosurgery integrated successfully without significant complications, providing sufficient bone volume for implant placement.[17] These clinical findings are further supported by in vitro studies demonstrating the presence of viable osteocytes and bone morphogenetic proteins in bone grafts harvested using piezosurgery.[18]

Piezosurgery offers a significant advantage in cystectomy, particularly when bone cutting is required near vital soft tissues, such as nerves and blood vessels, where the risk of mechanical or thermal injury must be minimized. One study reported that no nerve lesions occurred with piezosurgery, whereas surgery using rotary instruments resulted in reduced sensation lasting at least 1 week in 8% of patients.[19] These findings are consistent with the study by Schaeren et al., which demonstrated that, even under worst-case conditions, the direct exposure of a nerve to piezosurgery did not transect the nerve, but might result in limited structural or functional alterations.[9] In most cases, nerve regeneration can occur when the protective perineural sheath remains intact, unlike with conventional drills or oscillating saws. It was also observed that increased mechanical force applied to the nerve by the piezosurgery device resulted in greater tissue damage; however, this effect was not observed with ultrasonic vibration alone.[16]

The results of this study highlight a key advantage of piezoelectric instruments in the excision of cystic lesions, namely, the ability to perform precise ostectomy near delicate anatomical structures, such as the inferior alveolar nerve and the mental nerve, without causing damage. This advantage is attributed to the selective cutting mechanism of the device, which differentiates tissue hardness and acts primarily on mineralized structures. As the device automatically ceases cutting upon encountering non-mineralized tissues, it minimizes the risk of injury to soft tissues, including mucous membranes, nerves and blood vessels.[20]

The visual analog scale (VAS) scores for visibility indicate that ultrasonic surgery provides a significantly clearer surgical field as compared to conventional approaches, consistent with previous studies investigating ultrasonic surgery.[21] Unlike conventional micro-saws, in which blood accumulation within the cutting area may impair visibility, ultrasonic surgery maintains a nearly blood-free operative field during bone cutting procedures. This is achieved through the cavitation effect generated by the

distribution of the cooling fluid and the specific oscillation pattern of the instrument, which effectively clears blood from the surgical site and ensures optimal visibility throughout the procedure. Additionally, continuous saline irrigation prevents bone overheating, while intermittent micro-cavitation bursts contribute to hemostasis, resulting in a significant reduction in overall blood loss.[22]

The VAS scores for the ease of operation showed no significant differences between the piezosurgery and conventional surgery groups. Therefore, piezosurgery does not appear to provide a significant advantage in terms of improving operative ease as compared to conventional techniques. However, the frequent replacement of piezosurgery inserts and adjustments to machine settings, including power, oscillation and irrigation parameters according to the selected insert, may increase procedural complexity and operative time. Ongoing advances in piezosurgery technology are expected to address these limitations by introducing more user-friendly devices.[23]

The literature contains limited reports on the efficacy of piezosurgery in procedures involving soft tissues, such as cyst enucleation. However, several case reports and clinical studies have demonstrated favorable outcomes with the use of piezosurgery in the removal of jaw cysts,[24] cystic ameloblastoma,[13] and hyoid bone osteotomy during thyroglossal duct cyst surgery.[16] These findings suggest that piezosurgery may be an effective approach for managing these procedures.

Regarding surgical outcomes, ultrasonic surgery was found to be superior to conventional surgery in terms of intraoperative bleeding, epithelial perforation, postoperative complications, and recurrence. However, it should be noted that ultrasonic surgery was associated with a longer overall surgical duration. These findings are consistent with previous studies, which also reported increased operative time with the use of ultrasonic surgery.[16,23]

Although the effectiveness of the ultrasonic surgical approach in bone procedures has been consistently demonstrated, manual instruments remain necessary for certain soft tissue procedures and anatomically sensitive situations. In particular, manual instruments are preferred when operating around unerupted teeth in pediatric patients to prevent the potential displacement of tooth buds caused by ultrasonic oscillations from the surgical device. They are also favored when working near delicate anatomical structures, such as the orbital floor, during the the removal of large cystic lesions, as they allow surgeons to assess bone integrity through tactile feedback. Consequently, the development of new insert designs with different curves is needed to better accommodate these clinical requirements.[23]

Another drawback encountered during the use of ultrasonic surgery for cyst enucleation was insert breakage, with 2 inserts fracturing at their tips during procedures.[23] Several factors may contribute to insert failure, including sterilization processes, material fatigue due to repeated use, manufacturing defects, or the inappropriate selection of machine parameters during operation.[23]

The effectiveness of ultrasonic surgery depends on the precise manufacturing and design of the ultrasonic surgical device, including its piezoelectric crystals, electronic control circuits, handpiece materials, and working tips (inserts). Although piezoelectric bone surgery is relatively recent advancement in oral and maxillofacial surgery for bone-related procedures, substantial technological progress has been made in both device design and surgical techniques over the past decade.[24] Currently, several piezoelectric devices are available on the market, with newer versions continuing to be developed. It is important to recognize that similar piezoelectric units manufactured by different companies may demonstrate variations in performance. Furthermore, differences in insert design and performance may also influence clinical outcomes.[14]

The schematic comparison of piezosurgery and conventional surgery is presented in Fig. 3.

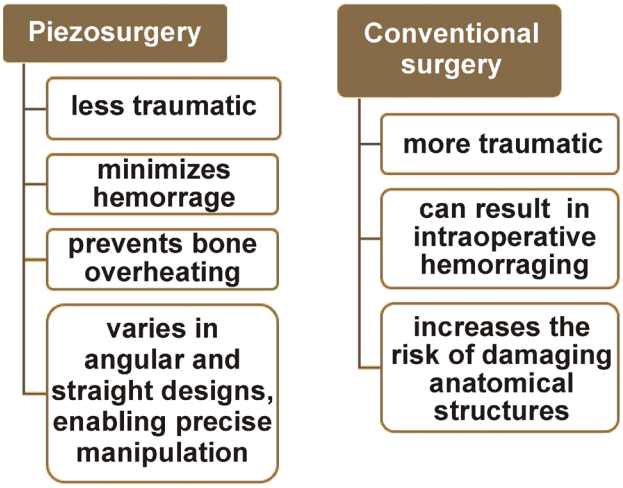


Fig. 3. Schematic comparison of piezosurgery and conventional surgery

Conclusions

Piezosurgery has demonstrated effectiveness in sensitive procedures, such as jaw cyst removal and enucleation, despite potentially requiring longer operative times. Compared with conventional methods using rotary instruments, piezosurgery provides significant reductions in postoperative pain and swelling. It may be a more favorable approach for cyst enucleation due to its ability to preserve important anatomical structures and provide improved surgical visibility. Additionally, piezosurgery may promote better early healing of the bone defect following enucleation. Although ultrasonic surgery for jaw cyst enucleation increases operative time, it significantly improves visibility, making it advantageous for delicate surgical manipulation and reducing the risk of injury to vital structures, including neurovascular tissues.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Review

Quorum sensing in chosen oral bacteria: A narrative review

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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

Quorum sensing (QS) is a communication mechanism enabling bacteria to inhabit countless habitats. Its role is mainly associated with the economization of gene expression, but also with gene transfers and biofilm development. There are 3 main QS patterns found in bacteria. This study examines intra-bacterial communication mechanisms and interactions between 3 key oral bacteria: *Streptococcus mutans*; *Staphylococcus aureus*; and *Porphyromonas gingivalis*. *Streptococcus mutans*, a Gram-positive bacterium, contributes to dental caries by forming robust biofilms via extracellular polysaccharides and QS. Contrastingly, *P. gingivalis*, a Gram-negative bacterium, is implicated in chronic periodontitis through its virulence factors like gingipains, and its role in dysbiotic biofilms. Both species adapt to oral cavity conditions and influence the host's immune response. Understanding QS in *S. aureus* is crucial due to its high prevalence among the general population and healthcare professionals, along with the commonness of antibiotic-resistant strains. The cited research on its accessory gene regulator (*agr*) system demonstartes its role in the high tolerance of *S. aureus* to environmental stress. Recognizing these mechanisms is crucial for developing strategies to manage oral health and tackle periodontal diseases. This research highlights the importance of bacterial biofilm, intra- and inter-species communication, and the adaptive strategies of these pathogens.

Keywords: quorum sensing, AHL, *agr*, autoinducer

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Highlights

- Quorum sensing (QS) is an important mechanism of regulation among bacteria.
- Mechanisms of QS vary between different species of bacteria.
- Quorum sensing is a promising target for antibacterial therapy.

Introduction

Bacterial pursuit toward dominating various environments requires a wide range of mechanisms, the most prominent being quorum sensing (QS), which draws resemblance to intracellular interactions in multicellular organisms.[1] Quorum sensing is at its core bacteria teaming up to achieve what is unachievable for a single cell. Essentially, the expression of each gene is modified according to the fluctuations of the population's strength and density. It could be argued QS is bacteria's way of optimizing the expenses in order to adjust to the ever-changing settings they find themselves in.

The present review highlights the intricate systems of intra-bacterial communication, which entail serious clinical implications. Realizing the scale and the commonness of QS may define the reasons why certain diseases, e.g. periodontitis, are so troublesome to combat, and with that in mind, new treatment possibilities arise, as QS transmitters may become targets for new chemotherapeutics.

Quorum sensing is mediated through a group of chemical molecules, which are produced by microorganisms and secreted to the surrounding environment. Historically, 3 types of QS molecules have been named: (1) oligopeptide autoinducers in Gram-positive bacteria; (2) acyl-homoserine lactones (AHLs), mostly found in Gram-negative bacteria; and (3) autoinducer-2 (AI-2) in both Gram-positive and Gram-negative species. These molecules mediate different types of QS, respectively (1) oligopeptide two-component type, (2) LuxI/LuxR-type, and (3) LuxS type.[2] Aside from that, other QS modules have been described, e.g. extracellular death factor (EDF) responsible for stimulating *mazEF*-regulated cell death in *Escherichia coli*.[3]

Intracellular coordination through QS makes it possible for microorganisms to regulate virulence factor production, biofilm formation, genetic information transfers, metabolic pathways in a cell, and more. To illustrate these processes, it is worth highlighting the following: biofilm development in *E. coli*, which can be modulated by AHLs in the SdiA-CsrB pathway[4]; surfactin (a QS molecule) deficiency in *Bacillus amyloliquefaciens*, which leads to a decrease in glycolysis and tricarboxylic acid cycle efficiency, and ultimately cell death[5]; an increase in the expression of motility-related genes (namely, *rhl*A, *rhl*B, *rhl*C) involved in the synthesis of rhamnolipids in *Pseudomonas aeruginosa* and conversion into the swarming phenotype in response to phosphate starvation[6]; or short-range QS that may be used to determine whether a horizontal gene transfer is necessary or possible, in other words, whether there is a suitable recipient cell within a sufficient distance from the donor.[7]

Quorum sensing is a supposedly perfect communication system – efficient, wide-spread among microorganisms and enabling collective behaviors. Despite the enormous amount of studies conducted on its role in prokaryota, it is by no means limited to that kingdom; eukaryota benefit from QS as much. *Candida albicans*, for example, produces farnesol involved in hyphal growth inhibition and promoting hypha-to-yeast switch.[8] What is more, bacterial QS molecules may interfere with *C. albicans* hyphal or biofilm formation. Certain diketopiperazines, 3-benzyl-6-isobutylidene-2,5-piperazinedione (QSSM 1157) and cyclo (L-Pro-L-Leu) (QSSM 1112), have been proven to successfully inhibit *C. albicans* dimorphism, thereby becoming a potent virulence inhibitor against the fungus.[9] On the other hand, symbiosis may occur between bacteria and fungi. The mortality associated with *Staphylococcus aureus* infection escalates from 80% to a dire 100% when *C. albicans* is present, as the latter boosts staphylococcal virulence factors (primarily alpha and delta-toxins) through its influence on the accessory gene regulator (*agr*) system.[10] It is worth mentioning that farnesol displays anti-cancer activity through the mechanism of increasing the apoptosis and decreasing the proliferation of cancerous cells, e.g. HCT-116 (human colorectal cancer), Saos-2 (human osteosarcoma) and human oral squamous cell carcinoma cells.[11,12]

Since QS has been shown to occur between different kingdoms, it should be obvious it is not merely an intra-species relationship; inter-species communication appears as well and as strongly. Much research has been conducted on the mechanisms in which other bacteria inhibit various aspects of *P. aeruginosa* virulence by hampering its QS systems, e.g. *Vibrio alginolyticus* secreting tyramine and N-acetyltyramine, which weaken pioverdine production,[13] or the *Delftia tsuruhatensis* extract hindering *P. aeruginosa* virulence on diverse levels, from biofilm formation, through motility, to virulence genes expression.[14]

The aim of this review was to exhibit 3 different QS mechanisms displayed by 3 of the bacteria species present in oral microbiota: *S aureus*; *Streptococcus mutans*; and *Porphyromonas gingivalis*. The research mainly focused on the molecular aspects of QS communication, aiming to identify common patterns across QS mechanisms and to highlight the clinical implications of QS in each bacterium.

A short word on biofilm

There is no biofilm without QS and there is little QS without biofilm. Repeated physical disturbances of biofilm may impede QS-regulated processes in pathogenic bacteria.[15] Biofilm formation model presented in 2002 by Sauer et al. is widely accepted, and provides a solid ground for further understanding of the problem.[16] At the 1st stage, singular cells attach to a glass surface, using specific structures (the study was conducted on *P. aeruginosa*, in which case the structure was the flagellum). The number of the attached cells grows and the development reaches its 2nd stage – the attachment becomes irreversible. That first layer, a foundation, is then covered with the cells clustering and building subsequent layers, reaching certain thickness milestones (described as maturation-1 and maturation-2), which mark stages 3 and 4. At the 4th stage, the bacterial cluster achieves its maximal dimensions. The final stage of biofilm development is dispersion, during which bacteria from the interior, central regions of the biofilm revert to the planktonic, motile state and leave the original cluster. While this pattern has been generalized to apply to many bacteria, including *S. aureus* (with slight nomenclature modifications introducing the stages of exodus and expansion between the previously described attachment and maturation stages),[17] it has been argued that this model does not fully capture the complexity of microbial communities. Similar to QS, polymicrobial biofilm-forming interactions have been described, for example, between *Vibrio cholerae* and *E. coli*.[18] Furthermore, biofilms are not always attached to solid surfaces. As demonstrated by Knott et al., free-floating aggregates of methicillin-sensitive *S. aureus* (MSSA) were isolated from the prosthetic joint infection fluid, highlighting the existence of unattached biofilms.[19] What is striking, the formations were more abundant and sophisticated than those formed by the same bacterium on a smooth, titanium surface.[19]

To fully fathom not only the process of biofilm formation, but also its overall advantages for the microorganism, insight into biofilm structure is crucial. It generally could be simplified as bacteria submerged in a gel-like fluid called extracellular matrix (ECM). The whole arrangement may be viewed as a "city of microbes"[20] representing the entirety of the biofilm with the microbes located in the "house of the biofilm cells", an accurate depiction of the matrix.[21] It is built of extracellular polymeric substance which contains polysaccharides, glycolipids, glycoproteins, certain amounts of extracellular DNA, and more. Establishing a comprehensive biochemical profile of ECM across the vast majority of biofilms remains unattainable. Nevertheless, several of its components have been characterized and shown to play essential roles in microbial biology. One such example is the proteinaceous component curli in *E. coli*, which contributes to biofilm structural integrity. This was demonstrated in a 3D-printed biofilm durability study that investigated the impact of curli presence or absence on biofilm resistance to chemical agents.[22] The authors showed that curli-positive biofilms remained intact following citrate treatment, and exhibited limited molecular oxygen penetration as compared to curli-negative biofilms printed under the same conditions.[22]

Biofilms might be formed by more than one microbial species. However, in such cases, QS occurs similarly to that observed in single-species aggregates. Such multispecies interactions may be more complex, and either competitive or cooperative in nature. A study by Armes at al. focused on 5 *Roseobacteraceae* strains exhibiting varied levels of QS component expression, ranging from paired LuxR/I QS systems in *Sagittula stellata* E-37, *Citreicella* sp. SE45 and *Rhodobacterales* strain Y4I to orphan LuxI and LuxR homologs in *Sulfitobacter* sp. EE-36 and *Roseovarius nubinhibens* ISM.[23] The study demonstrated that the Y4I strain possesses the ability to inhibit the growth of two of the 4 other strains, which directly corresponds with the indigoidine production observed in this strain. Indigoidine release is induced by the presence of metabolites produced by other community members within ECM, demonstrating its connection to a QS system in *phaR* and *phaI* mutants.[23] Another study investigating an in vitro model of a mixed-species biofilm formed by *P. aeruginosa* PAO1, *Pseudomonas protegens* Pf-5 and *Klebsiella pneumoniae* KP1 demonstrated that the biomass volume percentage of *K. pneumoniae* and *P. protegens* within the aggregate was dependent on the AHL-based QS system of *P. aeruginosa*.[24] The community containing QS-deficient *P. aeruginosa* exhibited a lower concentration of *K. pneumoniae* and a higher concentration of *P. protegens*, in contrast to the community containing wild-type *P. aeruginosa* with functional QS activity, which displayed a higher biomass volume of *K. pneumoniae* and a lower biomass volume of *P. protegens*.[24] These in vitro findings showcase the multispecies relations that might occur in every biofilm-rich environment, including human oral cavity, which confirms the intricacies of microbial interactions should be further researched.

The well-studied species revisited through the lens of their QS mechanisms are notorious biofilm producers with substantial clinical implications. The connection between *P. gingivalis* and periodontitis has long been recognized, with specific strains frequently identified among patients; for example, the W83_W50 strain was detected in the subgingival plaque samples obtained from 13% of participants in a recent study.[25] Furthermore, *Porphyromonas* spp. detected in the saliva samples of patients suffering from sialolithiasis have shown a positive correlation with *Porphyromonas* spp. present in their salivary stones.[26] Such a correlation was not observed for *Pseudomonas* spp.; however, another case report described the simultaneous recovery of *P. aeruginosa* and *P. gingivalis* strains from the bronchial sputum of a periodontal disease patient with a pulmonary abscess.[27] The authors suggested that this

infection may have resulted from the aspiration of these bacteria originating from the oral cavity.[27] These examples demonstrate that these bacteria remain sufficiently unpredictable to warrant further investigation, including a deeper understanding of their QS mechanisms.

Quorum sensing in *Staphylococcus aureus*

Staphylococcus aureus is a Gram-positive, facultative anaerobic, spheroidal bacterium usually occurring in clusters. Years of multidimensional research on this species provided a foundation for the current bacteriology knowledge. The significance of *S. aureus* is linked to its prevalence, among the general human population and notably healthcare professionals. A StaphDent study showed that 23.5% of the dentists tested carried MSSA,[28] while another study investigating methicillin-resistant *S. aureus* (MRSA) reported a 2.9% prevalence of these strains among dental personnel, with identification based on the presence of the *mecA* gene.[29]

The fact that *S. aureus* was present both in the outer and inner layers of the masks collected from dentists, dental assistants and nursing assistants, and made respectively 9.3% and 10.5% of the bacteria obtained from the samples prove the relevance of *S. aureus* in the context of this research.[30]

In line with other Gram-positive cocci, *S. aureus* possesses the two-component agr QS system. At the molecular level, it incorporates the RNAII and RNAIII transcripts, which are regulated by the P2 and P3 promoters, respectively.[31] RNAII is an operon consisting of 4 genes, *agrB*, *agrD*, *agrC*, and *agrA*, whereas RNAIII serves as the primary effector molecule of this regulatory system.[32] AgrC is a dimeric histidine protein kinase that undergoes autophosphorylation in response to the connection of the autoinducing peptide (AIP) to its receptor site.[33] The signal is subsequently transmitted to AgrA through phosphate group transfer.[31] The phosphorylated AgrA then binds to the aforementioned promoters and regulates the expression of RNAII and RNAIII, completing the QS regulatory loop.[34] Interactions between the cytosolic domain of AgrC and the response domain of AgrA are highly specific, as several conserved hydrophobic polypeptide sequences are present among *agr* types I–IV, with distinct differences as compared to other response regulators.[35] This relationship is highly significant, as mutant variants of the *agrB* transcript have demonstrated impairment in both protein stability and proteolytic activity – the former resulting in undetectable AgrD levels, while the latter leading to reduced AIP production in certain cases.[36] Regarding AIP itself, each molecule can be assigned to one of 4 different classes (I–IV) based on the sequence and length of the peptide chain, which ranges from 7 to 9 amino acids. Studies have shown that blocking AIP signaling through the removal of the peptides from the environment results in the inactivation of the *agr* QS system, leading to the inhibition of *agr*-dependent hemolysin expression.[37]

The mechanism has been tested thoroughly and each element of the *agr* system encodes various protective mechanisms, for example it protects *S. aureus* cells from lethal doses of H_2O_2, as mutants with deleted *agr* show lower rates of survival when exposed to reactive oxygen species (ROS).[38] Aside from that, cell death in a more propitious setting without any stressors is dependent on the *agr* system as well, since *agr*-absent cells show higher fractions of non-viable cells. However, deaths that may be related to *agr* occur in the *agr*-positive strains, in which case they facilitate the expansion of remaining, viable cells.[39] Regarding the regulation of the QS system, it is dependent not only on the expression products of the *agr* regulon, but also on other staphylococcal excretory factors. The P2 and P3 promoters rely heavily on AgrA for the activation of RNAII and RNAIII expression through a classical positive feedback mechanism; however, additional regulatory factors are also involved, including SarA and SarR, DNA-binding proteins belonging to the SarA family. The former stimulates P2 promoter activity, whereas the latter represses this process.[40] Another interesting aspect of *agr* regulation is the recent finding that androgens present in human skin, specifically testosterone and dihydrotestosterone, exert a measurable effect on the *S. aureus agr* transcriptome by activating the P3 promoter in a manner similar to AIP-I.[41]

Quorum sensing in *Streptococcus mutans*

This section on *S. mutans* is worth beginning by noting that this bacterium is relatively easy to culture under laboratory conditions, which has resulted in a considerable number of studies investigating its biology.[42]

S. mutans is a Gram-positive streptococcus characterized by high genotypic heterogeneity. A study by Cornejo et al. demonstrated that this bacterium possesses more than 3,000 genes, approx. half of which are conserved across all currently identified strains.[43] This genetic diversity plays an important role in competition among different *S. mutans* isolates. The considerable phenotypic variability of this species has also been highlighted.[44] *Streptococcus mutans* is classified as a lactic acid bacterium, meaning that it ferments carbohydrates and produces lactic acid. Furthermore, due to its high cariogenic potential, this bacterium can convert sucrose into extracellular polymers. Through the activity of glucosyltransferases and glycosyltransferases, among other enzymes, it produces water-insoluble glucans, which serve as a structural foundation for dental biofilm formation.[45]

It is worth noting that the etiology of dentin and root caries differs significantly, with *S. mutans* being primarily associated with the carious lesions affecting the tooth root. Research indicates that the prevalence of this condition is

substantial, with approx. 41% of individuals affected. This highlights the importance of further investigation into the communication mechanisms of this bacterium and its interactions with other members of the oral biofilm.[46]

In addition, *S. mutans* produces adhesins that interact with specific receptors, such as those present on the surface of the salivary pellicle. These adhesins also play an important role in the interactions of bacteria with each other within the biofilm. Among the most significant adhesins is antigen I/II. Studies have demonstrated that bacterial strains deficient in this component exhibit reduced ability to interact effectively with other cells, and consequently display lower cariogenic potential.[47–51]

Streptococcus mutans can efficiently adapt to the changing conditions of the oral cavity. It is capable of surviving under low pH conditions through a mechanism known as the acid tolerance response (ATR). This mechanism protects the bacterium from acid-induced damage[52–54]; as a result, the intracellular pH remains higher than that of the surrounding oral environment.[55]

However, the most relevant aspect of this article is the QS mechanism. The aforementioned mechanism is actively utilized by *S. mutans*, a member of the oral microbiota. A already note, *S. mutans* is also an important etiological agent of dental caries,[56] with its presence being associated with acidic saliva conditions.[57] Understanding how this bacterium employs QS may open new possibilities for developing methods to prevent dental caries.[58]

This type of inter-bacterial communication involves specific signaling molecules (pheromones) that can be detected by other bacteria. The vast majority of signals utilized in the QS mechanism are small-sized organic molecules. In some cases, bacteria also employ short-chain peptides consisting of up to 20 amino acids for this purpose.[59]

The description of the QS mechanism is worth beginning with its intra-species variant. Quorum sensing in *S. mutans* involves gene transcription processes that result in the production of compounds increasing the virulence of these bacteria. Furthermore, maintaining a self-perpetuating system, namely positive feedback, is essential; the factors responsible for this process are also generated during QS activation.[58] This system includes the AIP precursor, the transporter responsible for AIP export to the extracellular environment, and a histidine kinase, which initiates the transcriptional response.[58]

An inter-species model of QS signaling in *S. mutans* involves the use of AI-2, a class II signaling molecule that binds fish-like structures.[60–63] This signaling molecule is utilized by both Gram-positive and Gram-negative bacteria.[64] Its synthesis depends on the enzyme LuxS, which is essential, among other things, in the protein methylation pathway.[65] Once AI-2 is released into the extracellular environment, it is recognized by a specific receptor, initiating signal transduction, gene transcription and the production of virulence factors.[58]

Quorum sensing is important to mention in the context of the caries-forming potential of *S. mutans*, as this bacterium takes control of biofilm formation in the oral cavity. In addition, it also regulates the production of mutacins,[66,67] which adversely affect other bacteria in the surrounding environment.[56] Due to its genetic competence, *S. mutans* has the ability to acquire genetic material from other organisms present in the substrate and incorporate it into its genome. This allows it to acquire new traits or repair damaged DNA.[59] The greater the similarity between the DNA of the recipient cell and the DNA to be incorporated, the higher the probability of acquiring a new trait.[59] *Streptococcus mutans* uses type IV surface proteins for the aforementioned transformation mechanism.[68] The development of these competencies is most pronounced in only a few strains of *S. mutans*; however, compared with other streptococci, it remains relatively limited.[69] Increasingly, the literature suggests that competence is associated with the general bacterial response to stressors, as its activation is induced, among other factors, by mutagens and significant environmental fluctuations.[70]

Two major pathways are used for communication between *S. mutans* cells. They are presented below.

1. CSP–ComABCDE: a 5-gene system responsible for peptide export, enabling the stimulation of genetic competence in *S. mutans*.[71]
   The precursor peptide of CSP, a 21-amino acid competence-stimulating peptide, is ComC.[72] At an early stage, ComC is truncated by ComAB, resulting in the formation of the active CSP.[73] Accordingly, ComA can be considered to participate in CSP maturation.[74] The remaining genes, *com*D and *com*E, encode a histidine kinase and a response regulator protein, respectively.[75] Furthermore, the addition of CSP to non-competent cells has been shown to increase the frequency of genetic transformation.[76] This pathway is also crucial for the initiation of biofilm formation in the oral cavity,[77] as the ComDE system regulates, among other processes, the production of specific bacteriocins (mutacins) in *S. mutans*.[78]
2. XIP–ComRS.[56,58,79,80]
   Both cell-signaling pathways are interconnected. For the inducer peptide XIP, the precursor is ComS.[81] The ComR–XIP complex acts as a transcriptional activator of the *comR*, *comS* and *comX* genes. The activation of *comX* transcription ultimately leads to the development of genetic competence in *S. mutans*.[82]

Quorum sensing in *Porphyromonas gingivalis*

Porphyromonas gingivalis is a Gram-negative anaerobic bacterium that plays an important role in the development of chronic periodontitis, as it has the ability to induce the transition of the oral biofilm from a eubiotic to a dysbiotic

state.[83] Various virulence factors contribute to the pathogenicity of *P. gingivalis*, including the secreted lysine- and arginine-specific gingipains. These proteases are primarily associated with tissue destruction, but also contribute to the dysregulation of the host immune response.[84,85] Fimbriae are involved in adhesion to other bacteria and play an important role in biofilm formation.[86] *Porphyromonas gingivalis* has also been shown to possess extensive invasive potential, as it not only colonizes the oral cavity, but has also been detected in the aortic endothelium, where it is thought to gain access through the damaged epithelium.[87,88]

Numerous studies have been conducted to demonstrate the association between *P. gingivalis* and the occurrence of various diseases in the human body. Among other findings, they have linked the presence of this bacterium in periodontal disease with an increased risk of conditions such as myiasis, hypertension, gastrointestinal cancer, squamous cell carcinoma, Alzheimer's disease, and the development of diabetes-related complications.[89–95] These findings highlight the broad impact of *P. gingivalis* on human health. However, to fully understand its pathogenicity, it is worth beginning with the initial stage of this process, namely biofilm formation and, more specifically, the interactions among the bacteria that constitute the biofilm.

Three bacteria – *P. gingivalis*, *Tannerella forsythia* and *Treponema denticola* – constitute the so-called red complex. They can be detected in periodontal pockets, where they participate in the initiation and maintenance of the inflammatory process within the periodontium. These bacteria are considered the primary periodontal pathogens responsible for extensive destructive changes in periodontal tissues.[96] However, it is worth noting that the presence of a dental biofilm is a prerequisite for the *P. gingivalis* colonization of the gingiva.[97]

As previously mentioned, *P. gingivalis* is a Gram-negative anaerobic bacterium. It is highly capable of adapting to the conditions of the oral cavity, owing, among other factors, to its ability to adhere to the surfaces of the oral mucosa, cheeks and gingiva. This enables the bacterium to effectively resist the host's defense mechanisms.[98] *Porphyromonas gingivalis* exhibits extensive and highly diverse gene expression that supports its growth, maturation, survival, and communication with other bacteria within the dental biofilm.[98] The survival and function of *P. gingivalis* under these conditions are further facilitated by the presence of fimbriae and surface polysaccharides. These structures are key features promoting biofilm formation, as they enable the bacterium to adhere to the colonized surfaces and establish a highly specialized bacterial community.[99]

As mentioned earlier, QS is a fundamental mechanism of bacterial communication. Gram-negative bacteria produce specific QS signaling molecules – AHLs. It was initially believed that *P. gingivalis* did not produce these molecules; however, this assumption was later shown to be incorrect.[100] It is worth mentioning that AHL production has not been reported in other oral pathogens.[100–102] In a study by Muras et al., the authors indicated a probable association between the presence of AHLs in saliva samples and the formation of bacterial communities dominated by *P. gingivalis*, which was identified as the producer of these signaling molecules.[100] Interestingly, *P. gingivalis* possesses a homolog of the AHL synthase HdtS, and both AHLs and their homologous compounds have been shown to reduce the growth potential of this bacterium.[103] Furthermore, AHLs have the ability to alter protein expression in *P. gingivalis*.[100] The same study also reported that C8-HSL (N-octanoyl-L-homoserine lactone) was detected in saliva samples. The researchers observed that this compound was produced by *P. gingivalis*, a key periodontal pathogen. Notably, all individuals in whom C8-HSL was detected were diagnosed with periodontal disease.[100] In addition, *P. gingivalis* has been shown to produce AI-2, a furanosyl borate diester involved in the QS mechanism that enables communication with other bacterial species.[104]

In vivo findings on the reviewed bacterial species

It is worth noting that the aforementioned QS mechanisms are considerably more difficult to study in vivo because of the numerous interactions occurring within the microbial community, which may obscure the primary subject of investigation. Nevertheless, in vivo studies of QS remain a valuable source of knowledge about the overall phenomenon, and therefore should not be omitted from this review.

As the *agr* QS system should certainly be considered an important and relatively universal virulence factor of *S. aureus*, it may represent a potential target for antibacterial therapy. A compound that has shown promising results in in vivo studies is pyocyanin (PCN). The use of PCN against the 10 most virulent strains among 160 MRSA isolates, derived from the blood, wounds, sputum, and abscesses of patients admitted to an Egyptian hospital in a 2023 study by Abo Kamer et al., demonstrated a significant decrease in *agr*A gene expression in each tested strain.[105]

Streptococcus mutans QS is dependent on *Aggregatibacter actinomycetemcomitans* and the broader composition of the periodontal microbiota. The in vitro part of a 2017 study by Szafrański et al. demonstrated that the alternative sigma factor X (SigX) regulon, a central regulon of QS in *S. mutans*, is activated as a result of an inter-species QS-based interaction between these bacteria within a dual-species biofilm, as such activation does not occur in a single-species biofilm.[106] The in vivo part of the same study focused on this interaction, using samples derived from the periodontal pockets of 3 healthy patients and 1 patient suffering from chronic periodontitis. The results showed that SigX expression levels in the *S. mutans* strains isolated from periodontal pockets were similar to, and in

some cases higher than, those observed in in vitro dual-species biofilms.[106] Therefore, it could be argued that the presence of *S. mutans* within the multispecies environment of a periodontal pocket enables the expression of its virulence potential.

Porphyromonas gingivalis is a prominent target for anti-QS therapy; therefore, a number of studies investigating the suppressive effects of different compounds on its physiology have been conducted. For example, an experimental mouse model demonstrated that treatment with QS inhibitors (furan compounds and D-ribose) resulted in a decrease in bone destruction, measured as the distance between the cementoenamel junction (CEJ) and the alveolar bone crest (ABC), as well as a 68.36% reduction in *P. gingivalis* DNA levels as compared to the untreated, bacteria-infected group.[107] These findings further support QS inhibitors as a noteworthy research prospect in the context of periodontitis treatment.

Is there any correlation between the reviewed bacterial species?

A bacterial biofilm can exist primarily due to the cooperation and communication between microorganisms. Within this structure, the mutual aggregation of bacteria plays a decisive role. However, bacteria do not aggregate randomly; instead, there are specific patterns governing how this aggregation process occurs.[108] A good example is the aggregation of *S. mutans* with *Fusobacterium nucleatum* and the lack of interaction with *P. gingivalis*, as described earlier.[108] It is worth mentioning that the main mechanism underlying this process is the ability of bacteria to recognize polysaccharides located on their surfaces, which forms the basis of microbial communication. In addition, competition with other microorganisms for nutrient availability and favorable living conditions is also an important factor.[108]

An important element in the bacterial interaction chain is represented by proteins called bacteriocins. They are produced by numerous Gram-positive and Gram-negative bacteria; however, in the oral cavity, streptococci are the predominant producers of these compounds.[109] *Streptococcus mutans* produces 2 types of bacteriocins known as mutacins. They benefit this bacterium by effectively influencing its function and competitiveness within the biofilm.[110]

As previously mentioned, *S. mutans* does not exhibit aggregation with *P. gingivalis*, despite the presence of both microbial species being characteristic of patients with carious disease.[108] However, in the case of *S. mutans*, a correlation has been observed with another bacterium not described in the aforementioned article, but worth mentioning, namely *Streptococcus sanguinis*. Which bacterium dominates within a given environment depends on the order in which that environment is colonized.[108]

Furthermore, it was discovered that the CSP molecules involved in the communication pathway of *S. mutans* can be recognized by type 2 taste receptors (T2R). These receptors are proteins located on the membrane of host cells and can be found in the oral cavity, among other sites. Medapati et al. emphasized that *S. mutans* secretes QS molecules, namely the aforementioned CSPs, which are detected by these receptors.[111,112] Additionally, it has been demonstrated that CSP-1 is the most potent inducer of the QS molecule–receptor pathway in gingival epithelial cells.[113]

The research demonstrated the relationship that may exist between the aforementioned *S. mutans*, *S. aureus* and the described T2R receptor.[112] It showed that when the receptor was blocked, oral cavity cells exhibited greater difficulty in absorbing *S. aureus*, whereas the uptake of *S. mutans* remained unchanged. Additionally, when oral cavity cells were blocked with CSP-1, a similar effect was observed.[112]

Methods

A literature search was conducted using the PubMed and ScienceDirect databases, focusing on articles published up to the first half of 2024. The searched terms included keywords such as "quorum sensing mechanisms", "biofilm", species names of the described bacteria, and other related terms, often used in various combinations. The inclusion criteria required studies to be written in English, published in international peer-reviewed journals, and describing specific aspects of QS mechanisms among the investigated bacteria. To ensure the quality of the selected articles, the authors prioritized original research articles, with preference given to (1) pilot studies and (2) the most recent reports. Additionally, several case studies were referenced.

The final assessment of the presented review was conducted with the help of the Scale for the Quality Assessment of Narrative Review Articles (SANRA).[114] The authors evaluated the work according to 6 aspects: justification of the importance of the article (item 1); clear formulation of the aim (item 2); description of the methodology (item 3); referencing (item 4); scientific reasoning (item 5); and presentation of data (item 6). Each item was rated as 0 (low standard), 1, or 2 (high standard), with a maximum possible score of 12. Efforts were made to improve each element during the revision process in order to maintain the highest quality of reporting.

The key literature findings are presented in Table 1.

Limitations

Three bacterial species were selected, which represents a limitation, given the number of microorganisms present in the oral cavity. The complexity of the interactions occurring between these species is also a limitation, as it

Table 1. Key literature findings relevant to the present review

Reference number	Title	Year	First author	Aim of the work	Main conclusion for the review
16	*Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm	2002	Karin Sauer	Highlighting the dependence of *P. aeruginosa* physiology on the stage of biofilm formation as a useful biofilm growth tackling perspective	General biofilm formation pattern.
19	*Staphylococcus aureus* floating biofilm formation and phenotype in synovial fluid depends on albumin, fibrinogen, and hyaluronic acid	2021	Samantha Knott	Testing the bacterial susceptibility to changes in the concentration of aggregation-promoting proteins in the synovial fluid	Biofilm aggregates may be more complex when formed in the fluid medium than on a firm surface.
22	Emergent biological endurance depends on extracellular matrix composition of three-dimensionally printed *Escherichia coli* biofilms	2021	Srikkanth Balasubramanian	Displaying the relationship between the structure of the extracellular matrix and the endurance of a 3D-printed *E. coli* biofilm.	Curli-positive *E. coli* strains are more tolerant to harsh environments than curli-negative strains.
31	Finding of *agr* phase variants in *Staphylococcus aureus*	2019	Vishal Gor	Finding the possible cause for the lack or presence of Agr activity in *S. aureus*.	RNAII and RNAIII transcripts responsible for *S. aureus* QS are dependent on P2 and P3 promoters.
32	Conformational analysis and interaction of the *Staphylococcus aureus* transmembrane peptidase AgrB with its AgrD propeptide substrate	2023	Philip Bardelang	Investigating the interaction between the 2 elements of the *S. aureus agr* QS system – *agr*B and *agr*D – during the production of the autoinducing peptide.	RNAII consists of 4 genes – *agr*A, *agr*B, *agr*C, and *agr*D – crucial for *S. aureus* QS
37	Eliminating extracellular autoinducing peptide signals inhibits the *Staphylococcus aureus* quorum sensing *agr* system	2024	Ruki Inagaki	Investigating the effects of the removal of the autoinducing peptide from the *S. aureus* culture by a nitrocellulose membrane on QS-dependent virulence factor production.	The autoinducing peptide, as a QS mediator, is crucial for alpha-hemolysin expression in *S. aureus*.
58	Quorum sensing and quorum quenching with a focus on cariogenic and periodontopathic oral biofilms	2022	Patricia P. Wright	Discussing the principles and modern research on QS and quenching in cariogenic and periodontopathic biofilms.	Pheromones, which are mainly organic molecules or short-chain peptides, are involved in the QS mechanism.
68	Natural competence and the evolution of DNA uptake specificity	2014	Joshua Chang Mell	Investigating the specificity of DNA uptake by competent proteobacteria of the families *Pasteurellaceae* and *Neisseriaceae*, and presenting the mechanism of evolution and the stability of DNA uptake sequences.	The ComABCDE system, consisting of 5 genes, enables peptide export, stimulating the genetic competence in bacteria.
96	Mixed red-complex bacterial infection in periodontitis	2013	Nao Suzuki	Discussing the inter-species interactions of pathogens within the red complex, including *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*, in the context of their cooperative action in the destruction of periodontal tissues.	*P. gingivalis* is part of the red complex, which is often found in periodontal pockets and may be involved in the destruction of periodontal tissues.
98	Comparative gene expression analysis of planktonic *Porphyromonas gingivalis* ATCC 33277 in the presence of a growing biofilm versus planktonic cells	2019	María C. Sánchez	Investigating *P. gingivalis* gene expression in free-floating cells and monogeneous biofilms to better understand the microorganisms' adaptation to specific ecological determinants and their role in the biofilm environment.	*P. gingivalis* is capable of adhering to mucosal surfaces, enabling it to effectively evade host defense mechanisms, and communicate and cooperate with other bacteria within the oral biofilm.
100	Acyl homoserine lactone-mediated quorum sensing in the oral cavity: A paradigm revisited	2020	Andrea Muras	Investigating the presence of acyl-homoserine lactones (AHLs) in oral samples and their production by *P. gingivalis*, and evaluating potential strategies for AHL inhibition in the prevention and treatment of oral diseases.	*P. gingivalis* produces AHLs that are associated with the formation of bacterial clusters and pathogenicity in periodontal disease.
108	Bacterial interactions in dental biofilm	2011	Ruijie Huang	Discussing the main mechanisms of biofilm formation and interactions between oral bacteria.	*S. mutans* aggregates with *Fusobacterium nucleatum*, but shows no interaction with *P. gingivalis*.
112	Bitter taste receptor T2R14 modulates Gram-positive bacterial internalization and survival in gingival epithelial cells	2021	Manoj R. Medapati	Investigating the role of bitter taste receptors T2R14 in the internalization and growth inhibition of Gram-positive bacteria in gingival epithelial cells.	T2R receptors in the oral cavity recognize the competence-stimulating peptide molecules secreted by *S. mutans*, and by blocking them hinders the uptake of *S. aureus*, but does not affect *S. mutans*.

QS – quorum sensing.

introduces a potential risk of errors in the interpretation of QS mechanisms. Due to the complexity and broad scope of the topic, only selected mechanisms and molecules involved in QS were described.

Conclusions

The above examples cited in this article demonstrate how diverse, unique and complex the bacterial community inhabiting the human oral cavity is. It is not limited to simple communication, but represents a sophisticated system of information exchange that enables bacteria to cooperate and effectively inhabit all available niches. The examples presented illustrate the numerous directions, pathways and signals involved in QS mechanisms. The mechanisms of only 3 bacterial species have been described, while the oral cavity contains more than 700 bacterial species. Without cooperation, their survival would not be possible. Many of the pathways through which QS occurs have not yet been fully elucidated or thoroughly studied, leaving much more to be explored. Bacteria are highly capable of adapting to the prevailing conditions within the oral cavity.

Ethics approval and consent to participate

Not applicable.

Data availability

Not applicable.

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Use of AI and AI-assisted technologies

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Research letter

Vertical and horizontal dispersion of reference points and the reliability of the ANB, Wits, Tau, Yen, Sar, and W measurements: A repeatability study

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Abstract

Background. Various parameters are used in cephalometric diagnosis to assess the sagittal discrepancy of the maxillary bases.

Objectives. The aim of this study was to assess the role of the horizontal and vertical dispersion of anthropometric landmarks used for plotting, and to assess the reliability of the selected cephalometric parameters.

Material and methods. The material consisted of 24 randomly selected cephalometric radiographs. They were analyzed twice, 7 days apart, by 15 orthodontists. The horizontal and vertical dispersion (x, y) of individual anthropometric landmarks was assessed using the mean reference value, and the reliability of individual landmarks and measurements was assessed using the intraclass correlation coefficient (*ICC*(2.1)).

Results. The *ICC*(2.1) for each landmark ranged from 0.9907 to 0.9998. The *ICC*(2.1) for individual sagittal discrepancy measurements averaged 0.9370 to 0.9842. The highest reliability was achieved for the ANB, W, Sar, Wits, Tau, and Yen measurements, respectively. The obtained results indicate excellent reliability in determining landmarks. The measurements of the selected parameters assessing the sagittal incongruence relationship between the maxilla and the mandible also demonstrated excellent reliability.

Conclusions. The highest reliability in assessing the sagittal relationship between the maxilla and the mandible continues to be demonstrated by the ANB angle. The high and comparable values of the Sar, W, Tau, and Yen angles indicate the possibility of using these parameters interchangeably or complementarily in diagnosis and treatment planning, especially in borderline cases.

Keywords: orthodontics, algorithms, reproducibility of results, statistics and numerical data, cephalometry

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Highlights

- Landmark dispersion affects the accuracy of cephalometric diagnosis.
- Horizontal landmark dispersion plays a more significant role in the assessment sagittal discrepancy.
- The high reliability of landmark identification and cephalometric measurements supports the use of ANB, Wits, Sar, W, Tau, and Yen measurements for diagnosis and orthodontic treatment planning.
- When applying the new diagnostic measurements, particular caution should be exercised in patients undergoing growth and development.
- A major limitation of the new cephalometric measurements is their inability to determine whether the maxilla or mandible is affected by abnormal development.

Introduction

The method of choice for precisely assessing the sagittal relationship between the maxilla and the mandible[1–23] is cephalometric analysis. Since the 1930, when Broadbent and Hofrath introduced cephalometry,[4] the number of new cephalometric analyses based on new landmarks has been steadily increasing. One reason for this is the desire to find methods that are least susceptible to disturbances that could affect the measurement results. The authors of these analyses[5,7,8,12,19] indicate that the anthropometric points they proposed, which constitute the basis for obtaining measurements, are less sensitive to changes in the skeletal structure related to growth[2–7,9] or completed orthodontic treatment[2–15] than the previously used ANB,[11,13] Wits,[11] Beta,[13] μ,[16] ODI (Overbite Depth Indicator),[2] and APDI (Anteroposterior Dysplasia Indicator)[2] measurements. In a systematic review from 2022, the authors identified several new angular measurements (Tau, Yen, Sar, W) based on the new landmarks G, M, W, and T.[2]

The reliability (i.e., accuracy) of individual Cartesian measurements along the 0X and 0Y axes depends on the repeatability and reproducibility of landmark identification by orthodontists performing the examination. A measurement is considered reliable when it is both precise (repeatable) and consistent across the examiners or repeated assessments (reproducible). In practical terms, reliability is reflected by the overlap of the landmark positions identified by the examiners on the Cartesian coordinate system, or, when perfect overlap is not achieved, by minimal dispersion of the identified points.

Previous studies conducted by Kotuła et al.[2–4,15–17] did not demonstrate increased measurement precision with the use of the newly proposed landmarks. Of these, only one study[3] evaluated the reliability of the horizontal and vertical dispersion of the landmarks used for the ANB and Tau measurements. The results indicated that clinicians' knowledge and proficiency in landmark identification play a significant role in improving measurement reliability.

To minimize the limitations of single measurements, especially in borderline cases, clinicians combine parameters from selected analyses and compare them to precisely assess sagittal discrepancy.

The aim of this study was to assess the role of the horizontal and vertical dispersion of 12 anthropometric landmarks. The impact of point identification on the reliability of the Tau, Yen, Sar, W, and Wits angle measurements was assessed in correlation with the ANB angle, considered the "gold standard" (Table 1).

Table 1. Reference points in radiographic cephalometry included in the analysis

Point	Description
A	the deepest point of the anterior maxillary concavity, between the anterior nasal spine and the alveolar crest
B	the deepest point of the anterior mandibular concavity, between the alveolar crest and the pogonion
N	the most anterior point of the nasal-frontal suture on a radiographic profile
S	the center of the sella turcica
G	the center of the circle tangent to the inner lower, anterior and posterior surfaces of the mandibular symphysis
M	the center of the premaxilla
W	the mean intersection point of the lower contours of the anterior scalene processes and the contour of the anterior wall of the sella turcica
T	the highest point at the junction of the anterior wall of the pituitary fossa and the tubercle of the sella turcica
Iss	the most inferior and anterior point on the incisal edge of the maxillary central incisor
Iis	the most superior and anterior point on the incisal edge of the mandibular central incisor
U6	the most posterior point on the crown surface of the maxillary first molar
L6	the most posterior point on the crown surface of the mandibular first molar

Material and methods

Ethical approval

The study was approved by the Bioethics Committee of the District Medical Chamber (decision 01/173/2023 of March 6, 2023). Written informed patient consent was obtained in accordance with the Declaration of Helsinki.[24]

Inclusion and exclusion criteria

The following inclusion criteria were established: generally healthy Caucasian patients aged 12–18 years; prior to orthodontic treatment; without developmental defects; with the cephalograms taken at the neutral head position (NHP).

The following exclusion criteria were applied: cephalograms with anatomical abnormalities that hindered the correct identification of landmarks; cephalograms of individuals with significant craniofacial deformities; and cephalograms showing the displacement of bilateral anatomical structures relative to each other.

Characteristics of methods

The sample size (N = 24) was determined assuming a significance level of $\alpha = 0.05$, an effect size (f) of 0.60, and a statistical power of 80% ($1 - \beta = 0.80$). The study included 24 digital lateral cephalograms acquired using a two-dimensional (2D) imaging technique, with 8 radiographs representing each of the 3 skeletal classes. The radiographs were randomly numbered from 1 to 24 and entered into an Excel spreadsheet (Microsoft Corp.; Redmond, USA).

Cephalometric analysis was performed based on the manually identified reference landmarks, using the Ortodoncja software, v. 9.0 (Ortobajt, Wroclaw, Poland), and certified high-resolution NEC MultiSync EA244WMi monitors (NEC, Tokyo, Japan). The monitor specifications included a 24-inch display, a resolution of 1920 × 1200 pixels, a pixel pitch of 0.270 mm, horizontal and vertical viewing angles of 178°, and a contrast ratio of 1000:1. Landmark identification was performed manually on the digital images using a computer mouse. Before analysis, each image was calibrated by one investigator (J.K.) using the 3-centimeter reference ruler available in the software calibration window.

All radiographs were anonymized, randomly coded from 1 to 24, and the orthodontists were blinded to both the image identities and their previous assessments. The anonymized images were distributed to 15 dentists undergoing postgraduate orthodontic training, each with at least 2 years of experience in cephalometric image analysis. After completing the evaluations, the coded datasets were returned to the study supervisor, entered into Excel spreadsheets, and subjected to statistical analysis.

Each examiner performed the cephalometric analyses of the ANB, Wits, Tau, Yen, SAR, and W measurements twice, with a 7-day interval between the assessments. In total, 180 cephalometric analyses were completed, involving the identification of 2,160 anthropometric landmarks and 4,320 measurements along the X- and Y-axes. Overall, 8,640 Cartesian coordinates were analyzed.

The "gold standard" was used only as a reference point for coordinate normalization, not as an absolute diagnostic truth. Its value was defined as the average of the coordinate points identified by the 2 principal investigators (J.K and K.K.). The principal investigators analyzed 1,152 coordinate points. The agreement between them in classifying 24 radiographs into one of the 3 skeletal classes was verified by Fleiss's kappa coefficient ($\kappa = 0.999$). This value indicates excellent inter-individual agreement.

Statistical analysis

Statistical analyses were performed using Statistica, v. 13.3 (TIBCO Software Inc., Palo Alto, USA). The reliability of the horizontal and vertical landmark coordinates, as well as the cephalometric measurements, was assessed using the two-way random-effects intraclass correlation coefficient for absolute agreement (*ICC*(2.1)). The *ICC* values were interpreted according to the guidelines proposed by Koo and Li.[4]

Results

The accuracy of determining the Cartesian coordinates of the 12 cephalometric landmarks determines the reliability of estimating the indices that assess the mutual relationship between the maxilla and the mandible in the sagittal plane.

To accurately assess the location of each of the 12 landmarks on each of the 24 cephalograms included in the study, a reference value was established for each landmark. The reference value was defined as the mean of duplicate measurements of each cephalometric point performed by 2 experts. This reference standard was expressed as Cartesian coordinates (x, y) for each landmark relative to a fixed reference point on each image. The coordinates obtained from each measurement were normalized by subtracting the corresponding reference coordinates for each patient from the measured values.

The *ICC*s were used to quantify the horizontal and vertical dispersion of individual landmarks.

The dispersion of individual landmarks was assessed by comparing the obtained measurements within the corresponding ranges of the identified points at $p < 0.001$ and a 95% confidence interval (*CI*) (Fig. 1).

Relative to the X-axis, the Nx and Sx coordinates were identified with the highest precision (*ICC*(2.1) = 0.9998; 95% *CI*: 0.9984–0.9999), whereas the Tx coordinate showed

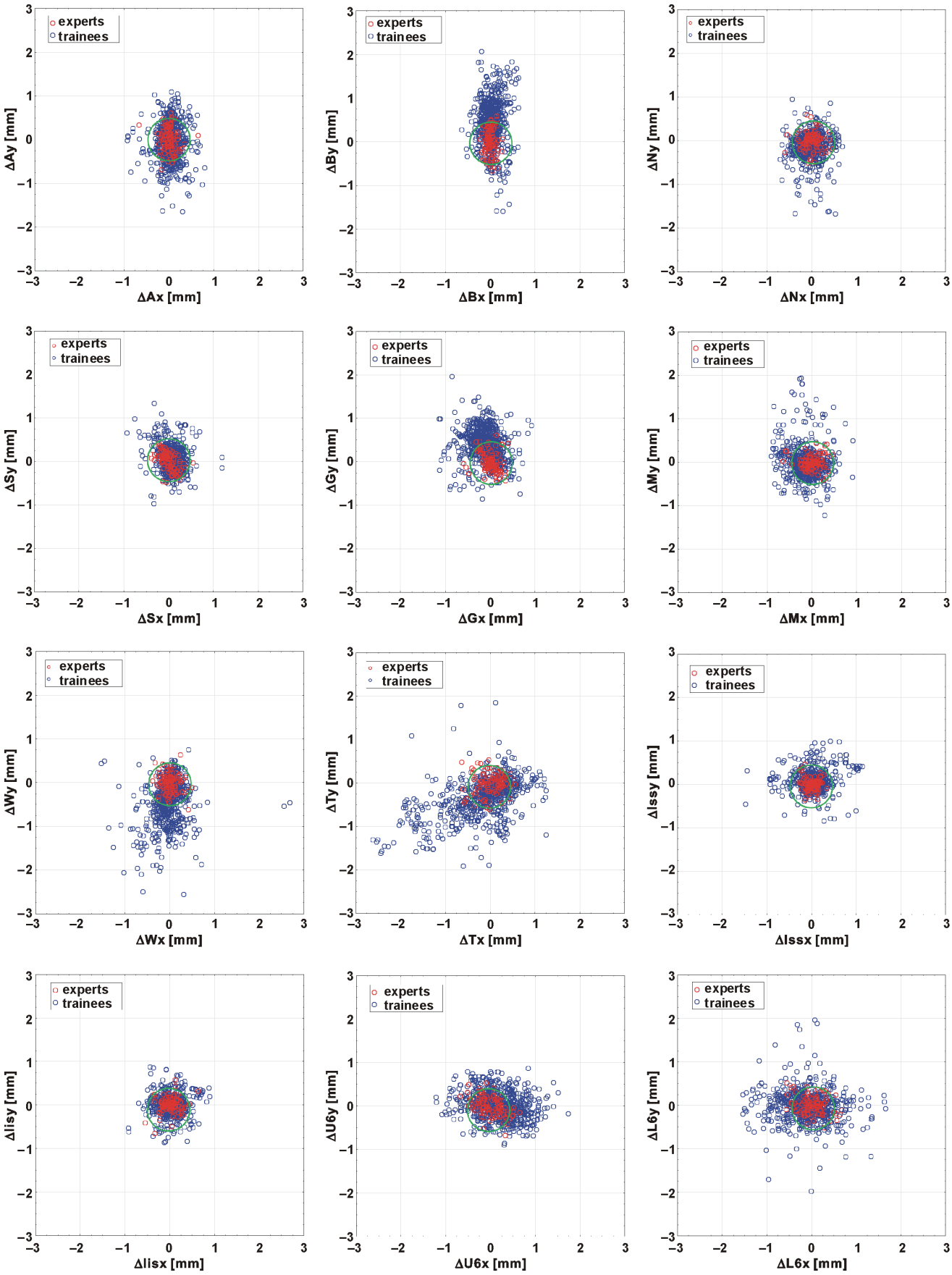


Fig. 1. Scatter plot of the cephalometric landmarks identified by 2 experts (red dots) and 15 trainees (blue dots). The tolerance zone is represented by the green circle with a radius of 0.5 mm

the lowest precision (*ICC*(2.1) = 0.9970; 95% *CI*: 0.9938–0.9986) . The horizontal precision of the newly introduced landmarks was as follows: Gx, *ICC*(2.1) = 0.9994 (95% *CI*: 0.9987–0.9999); Mx, *ICC*(2.1) = 0.9994 (95% *CI*: 0.9983–0.9998); Wx, *ICC*(2.1) = 0.9994 (95% *CI*: 0.9984–0.9998); and Tx, *ICC*(2.1) = 0.9970 (95% *CI*: 0.9938–0.9986). Among the newly introduced landmarks, Tx was identified with the lowest horizontal precision (*ICC*(2.1) = 0.9970; 95% *CI*: 0.9938–0.9986.

Relative to the Y-axis, the Ny coordinate demonstrated the highest precision (*ICC*(2.1) = 0.9988; 95% *CI*: 0.9978–0.9992), whereas the By coordinate showed the lowest precision (*ICC*(2.1) = 0.9865; 95% *CI*: 0.9770–0.9932). The vertical precision of the newly introduced landmarks was as follows: Ty, *ICC*(2.1) = 0.9967 (95% *CI*: 0.9958–0.9975); Wy, *ICC*(2.1) = 0.9958 (95% *CI*: 0.9948–0.9970); My, *ICC*(2.1) = 0.9955 (95% *CI*: 0.9945–0.9965); and Gy, *ICC*(2.1) = 0.9907 (95% *CI*: 0.9900–0.9910). Among these landmarks, By was identified with the lowest vertical precision (*ICC*(2.1) = 0.9865; 95% *CI*: 0.9770–0.9932).

The overall mean reliability of landmark identification was *ICC*(2.1) = 0.99 (95% *CI*: 0.98–0.99).

The Bland–Altman plots demonstrated high repeatability of the measurements, with angular parameters showing a mean difference of approx. 0.01° and the limits of agreement ranging from –1° to 1°, while linear measurements showed a mean difference of approx. 0.01 mm and the limits of agreement ranging from –0.75 to 0.75 mm, indicating high measurement reliability.

The highest reliability for sagittal discrepancy measurements was observed for the following parameters: ANB, *ICC*(2.1) = 0.9842 (95% *CI*: 0.98–0.99); W, *ICC*(2.1) = 0.9700 (95% *CI*: 0.97–0.98); Sar, *ICC*(2.1) = 0.9664 (95% *CI*: 0.96–0.97); Wits, *ICC*(2.1) = 0.9576 (95% *CI*: 0.95–0.96); Tau, *ICC*(2.1) = 0.9533 (95% *CI*: 0.95–0.96); and Yen, *ICC*(2.1) = 0.9370 (95% *CI*: 0.93–0.95). Among the evaluated parameters, the ANB angle demonstrated the highest measurement precision (*ICC*(2.1) = 0.9842; 95% *CI*: 0.98–0.99), whereas the Yen angle showed the lowest precision (*ICC*(2.1) = 0.9370; 95% *CI*: 0.93–0.95).

As an additional measure of landmark localization accuracy, we calculated the proportion of points located within the predefined tolerance zone. The tolerance zone was arbitrarily defined as a circle with a radius of 0.5 mm centered on the reference point. Localization accuracy was expressed as the ratio of the number of points falling within the tolerance zone to the total number of evaluated points.

Among the measurements performed by the experts, most landmarks (S, M, L6, W, U6, G, and T) were identified with greater precision in the vertical dimension, whereas the N, A and B landmarks were identified more precisely in the horizontal dimension.

Among the measurements performed by the residents, most landmarks (Iis, S, N, A, W, G, and B) were identified with greater precision in the horizontal dimension. In contrast, the Iss, M, L6, U6, and T landmarks were identified with greater precision in the vertical dimension.

Among the experts, the probability of correct landmark identification in both dimensions was highest for the following points, in descending order: Iss, S, Iis, W, M, N, A, B, G, U6, L6, and T. Among the physicians participating in the course, the corresponding order was: Iis, S, N, Iss, M, A, L6, W, U6, G, T, and B.

In the group of 15 trainees, the Iis landmark was identified with the highest accuracy (89.4%), whereas the B landmark showed the lowest accuracy (47.4%). The reduced accuracy of the B landmark was primarily attributable to errors in vertical localization (By). Despite this, the reliability of the ANB angle was not substantially affected.

Discussion

Cephalometric analysis is one of the key components of the diagnostic process preceding orthodontic treatment planning.[1–23,25–29] Following the systematic review by Kotuła et al. published in 2022,[2] numerous studies have systematically investigated reliable methods for assessing sagittal skeletal discrepancy.[3–10,12,14,15,18] In the present study, the authors continued these efforts by evaluating the reliability of the ANB, Wits, Tau, Yen, Sar, and W parameters.

Other authors have also commented on these angles. Babbar et al., who evaluated the ANB, Wits, Yen, W, and Pi measurements, demonstrated that all of these parameters can be used to assess the sagittal skeletal relationship.[20] In borderline cases, however, they recommended confirming the diagnosis using alternative measurements.[20] Kotuła et al. reported that the errors affecting the reliability of sagittal discrepancy measurements, particularly in growing patients, are closely related to accurate landmark identification.[3] The positions of landmarks A, N and B may change considerably during growth and throughout orthodontic treatment, complicating the accurate assessment of sagittal discrepancy. In particular, the horizontal dispersion of landmarks A and B relative to the X-axis, together with the vertical dispersion of landmark N relative to the Y-axis, is critical for the reliability of the ANB measurements.[3]

In assessing the reliability of the Tau angle, the horizontal dispersion of landmarks T and M relative to the X-axis, as well as the vertical dispersion of landmark G relative to the Y-axis, are of particular importance.

In the present study, the *ICC* values for the horizontal dispersion of landmarks A, N, B, T, M, and G were all greater than 0.997. The *ICC* value for the vertical dispersion of landmark N (0.9988) indicated excellent agreement between the measurements. Although landmark B exhibited the lowest agreement for vertical localization (By), with an ICC of 0.9865, this did not substantially reduce the reliability of the ANB angle for assessing sagittal discrepancy.

Kotuła et al. noted that the mean differences between the repeated measurements obtained by the same assessor for the ANB, Tau, and Yen angles were minimal.[4] However, the wide range of the 95% limits of agreement between measurements remained acceptable. The reliability analysis demonstrated a high level of intraclass agreement.[4]

Of particular importance is the potentially reduced susceptibility of points G and M to changes associated with growth or orthodontic treatment. This hypothesis was supported by the study of Gupta et al.[9] and challenged by the findings of Kotuła et al.[4,16] Therefore, it cannot be conclusively stated that the Tau angle is more resistant to size-related changes associated with mandibular growth rotation.

The results of the study by Khushal et al. indicate that the ANB angle requires critical interpretation, taking into account the patient's age, maxillary growth rotation, vertical growth pattern, and anterior cranial base length.[18] The authors note that the position of the N landmark is not stable throughout growth. Moreover, growth-related changes or orthodontic treatment may alter the positions of landmarks A and B, consequently affecting the ANB measurement. The authors emphasize that excluding the unstable N landmark from the Wits measurement does not eliminate the possibility of measurement errors. The occlusal plane used for this assessment is susceptible to changes resulting from missing teeth or the stage of tooth eruption. These factors may alter the angle and inclination of the occlusal plane, thereby affecting the relative positions of landmarks A and B and, consequently, the Wits measurement itself.[18]

In the evaluation of measurement reliability across 2 measurement sessions performed by the same orthodontists, no systematic reduction in errors or increase in agreement was observed in the second series of measurements. This finding indicates the stability of landmark identification skills within the research group.

The current research confirms the conclusions of Khushal et al.[18] regarding the reliability of the W angle measurement.

Limitations

The study has several limitations: a small number of patients and investigators; the lack of gender differentiation; the lack of a comparative analysis using a longitudinal study; and the use of a single system for cephalometric analyses.

This study is of a pilot nature, and the confirmation of the obtained results requires a longitudinal investigation, particularly in patients undergoing growth and development.

Conclusions

The horizontal and vertical dispersion of landmarks used for the assessment of sagittal discrepancy influences the accuracy of cephalometric diagnosis. The present findings demonstrate the high reliability of the ANB, W, Sar, Wits, Tau, and Yen measurements, supporting their careful application in orthodontic diagnosis and treatment planning, particularly in growing patients.

High-quality lateral cephalometric radiographs contribute to the reliable assessment of landmark localization. To further improve measurement reliability, clinicians should receive appropriate training in landmark identification. In less experienced practitioners, horizontal landmark dispersion appears to play a more significant role in the assessment of sagittal discrepancy.

A major limitation of the Tau, Yen, Sar, and W measurements is their inability to determine which jaw is affected by abnormal development. Therefore, the assessment of their validity requires correlation with SNA and SNB angle analyses.

The limitations of the present study require careful interpretation of the evaluated parameters.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee of the District Medical Chamber (decision 01/173/2023 of March 6, 2023). Written informed patient consent was obtained in accordance with the Declaration of Helsinki.[24]

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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Research letter

Botulinum toxin injections into masticatory muscles: A brief review on applications in dental occlusion management for orthognathic surgery and facial fractures

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Abstract

Background. Orthognathic and maxillofacial trauma surgeries are often challenged by postoperative instability resulting from persistent masticatory muscle forces acting on the repositioned bone segments. Botulinum toxin type A (BTX-A) has been proposed as an adjunctive intervention to transiently reduce these forces and enhance surgical stability.

Objectives. The aim of this narrative review was to evaluate the adjunctive role of BTX-A in orthognathic and maxillofacial trauma surgery, with particular emphasis on its potential to reduce postoperative masticatory muscle forces, thereby enhancing skeletal stability and optimizing healing. The review summarizes the current evidence regarding target muscles, dosing strategies, timing of administration, effects on skeletal and occlusal outcomes, and potential impact on bone regeneration. In addition, it identifies limitations in the existing clinical protocols and highlights areas requiring further standardized, high-quality research.

Material and methods. A narrative review was conducted using the PubMed, Scopus, and Web of Science databases to identify experimental, clinical and case-based studies investigating the use of BTX-A in orthognathic surgery and maxillofacial trauma management. The reference lists of relevant articles were also manually screened to identify additional key mechanistic and translational evidence.

Results. Botulinum toxin type A injections into the masseter, temporalis and digastric muscles may reduce postoperative muscle tension, protect fixation hardware, and improve skeletal and occlusal stability. The available studies suggest a transient 20–40% reduction in bite force lasting approx. 3–4 months, corresponding to the critical period of bone healing. Experimental models also indicate a potential beneficial effect on bone regeneration.

Conclusions. Botulinum toxin type A represents a minimally invasive and biologically plausible adjunct for enhancing outcomes in orthognathic and maxillofacial trauma surgery. However, standardized protocols regarding dosing, timing of administration, and safety have yet to be established. Well-designed controlled clinical trials are needed to define its optimal use, alongside ethical guidance addressing its off-label application and use in pediatric patients.

Keywords: facial trauma, orthognathic surgery, masticatory muscles, botulinum toxin type A, occlusal stability

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Highlights

- Botulinum toxin injections reduce postoperative masticatory muscle forces, supporting skeletal stability in orthognathic and maxillofacial trauma surgery.
- The adjunctive use of botulinum toxin reduces the risk of fixation hardware failure and skeletal relapse.
- Botulinum toxin may enhance bone healing and remodeling by modulating muscle tension and the local biomechanical environment.
- Botulinum toxin shows clinical utility in managing challenging cases, including condylar fractures, deep bite relapse and hyperdivergent skeletal patterns.
- Since botulinum toxin is currently used off-label in maxillofacial surgery, there is a need for standardized dosing protocols and ethical guidance.

Introduction

Orthognathic surgery and maxillofacial trauma management frequently involve the repositioning of the facial skeleton to correct occlusal or esthetic discrepancies. Despite advances in rigid internal fixation and three-dimensional (3D) surgical planning, postoperative complications such as skeletal relapse, malocclusion and hardware failure remain significant challenges, particularly due to variability in planning accuracy and postoperative biomechanical adaptation.[1,2] These complications are often attributable to the persistent forces exerted by the masticatory and suprahyoid musculature during the healing period.[3]

Botulinum toxin type A (BTX-A), a neurotoxin derived from *Clostridium botulinum*, has emerged as a potential adjunctive therapy in maxillofacial surgery due to its ability to induce temporary muscle paralysis.[4] The injection of BTX-A into the masticatory muscles is well established in the management of temporomandibular disorders (TMD)[5–8] with a predominantly myogenous component[9,10] and in the treatment of bruxism-related symptoms.[11,12] By transiently weakening hyperactive or mechanically disadvantageous muscles during the critical postoperative healing phase, BTX-A may reduce the undesired movement of osseous segments, minimize complications, and promote more predictable occlusal and skeletal outcomes.[13–16]

Although BTX-A has been extensively investigated for orofacial pain,[4,17] TMD[9,18] and cosmetic indications,[19] its adjunctive application in orthognathic and maxillofacial trauma surgery remains relatively underexplored, with the available evidence dispersed across case reports, experimental studies and small clinical series. Furthermore, the literature lacks a comprehensive synthesis focused on its potential role in biomechanical stabilization, postoperative occlusal control and the protection of fixation constructs. This narrative review addresses this gap by critically summarizing the available experimental and clinical evidence regarding BTX-A injections into the masticatory and suprahyoid muscles as a surgical adjunct, with particular emphasis on the mechanisms of action, clinical indications, dosing variability, ethical considerations, and current limitations.

To our knowledge, this is the first narrative review specifically dedicated to the use of BTX-A as a biomechanical adjunct for enhancing occlusal stability and protecting fixation constructs in orthognathic surgery and maxillofacial trauma management, integrating mechanistic, clinical and ethical perspectives.

Methods

Study design

This narrative review evaluates the clinical and experimental evidence regarding the use of BTX-A injections into the masticatory and suprahyoid muscles to enhance occlusal stability and bone healing following orthognathic surgery and maxillofacial trauma. The review synthesizes current mechanistic insights, clinical outcomes, and the emerging considerations related to dosing, safety and accessibility.

Search strategy, data extraction and synthesis

A comprehensive electronic search was conducted in the PubMed/MEDLINE, Scopus, and Web of Science databases for studies published up to September 2025, without language restrictions. The search strategy combined the following keywords and Medical Subject Headings (MeSH) terms: “botulinum toxin” OR “BTX-A” AND (“orthognathic surgery” OR “jaw surgery” OR “maxillofacial fracture” OR “facial trauma” OR “masticatory muscle” OR “occlusal stability”). The reference lists of all included articles and relevant review papers were also manually screened to identify additional eligible studies.

Data extraction was performed independently by two reviewers (MV and MG) using a predefined, standardized data extraction form. For each included study, the following variables were systematically collected: first author and year of publication; study design; clinical indication and patient characteristics; sample size; type of botulinum toxin administered; dosage and dilution protocol; anatomical

injection sites; timing of injection in relation to surgery or trauma; presence and type of a comparator group; duration of follow-up; primary clinical and radiographic outcomes; findings related to relapse or postoperative stability; and reported adverse events.

Particular attention was paid to variables directly related to occlusal stability and biomechanical unloading, including the targeted muscles (e.g., masseter, temporalis, digastric, and pterygoid muscles), timing of injection (preoperative, intraoperative or postoperative) and outcome measures used to assess relapse (e.g., changes in overbite, cephalometric parameters, and hardware failure). When quantitative data were incomplete or reported heterogeneously, findings were synthesized narratively to preserve their clinical relevance. Outcome heterogeneity was further influenced by differences in the assessment methods, including conventional cephalometry, cone-beam computed tomography (CBCT)-based analysis and digital model evaluation, which have been shown to produce non-equivalent results across studies.[20]

Disagreement at any stage of study screening or data extraction was resolved through discussion, with consensus reached in all cases without the need for third-party arbitration. Although this study was designed as a narrative review, a structured screening process informed by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) principles was adopted to enhance transparency and reproducibility. Studies were excluded following full-text review if they lacked relevance to surgical applications, focused exclusively on cosmetic indications, or did not report outcomes related to occlusal or skeletal stability.

Inclusion and exclusion criteria

Eligible studies included: (1) experimental or clinical investigations evaluating the use of BTX-A in orthognathic surgery or maxillofacial trauma contexts; (2) studies reporting injection sites, dosage, or outcomes related to occlusal or skeletal stability; and (3) animal studies providing mechanistic or histological evidence with potential translational relevance.

The exclusion criteria comprised: (1) studies focusing exclusively on esthetic or cosmetic indications; (2) investigations of orofacial pain, bruxism or TMD without a surgical context; and (3) reviews, editorials or opinion articles lacking original data.

Risk of bias assessment

Given the predominance of non-randomized studies and case-based reports in the included literature, the risk of bias was assessed using a qualitative approach adapted to the design of each study.

For non-randomized comparative studies, the risk of bias was evaluated according to the principles of the Risk of Bias in Non-randomized Studies-of Environmental Exposure (ROBINS-E) tool,[21] with particular consideration given to bias arising from confounding, participant selection, deviations from the intended interventions, and outcome measurement. For uncontrolled case series and case reports, methodological quality was assessed based on the completeness of clinical descriptions, the clarity of intervention protocols, the adequacy of follow-up, and the transparency of outcome reporting.

Studies were categorized as presenting moderate, moderate-to-high, or high risk of bias, without the application of numerical scoring systems, given the heterogeneity of study designs and outcome measures. This qualitative classification was considered more appropriate for reflecting the exploratory and hypothesis-generating nature of the available evidence.

Results and discussion

The electronic search was completed on September 30, 2025. The database search identified 86 records from PubMed/MEDLINE, 414 from Scopus, and 203 from the Web of Science. Following duplicate removal, 54 records were screened based on title and abstract evaluation. Twenty-three full-text articles were assessed for eligibility, and 9 studies[3,13–15,22–26] were included in the qualitative synthesis. Table 1 summarizes the included studies and their main characteristics.

Unless otherwise specified, claims derived from single case reports are presented descriptively and should not be interpreted as the evidence of efficacy comparable to controlled clinical studies.

Quality of evidence and the strength of clinical claims

The overall quality of evidence supporting the adjunctive use of BTX-A in orthognathic and maxillofacial trauma surgery remains low to moderate.[13,22,23] The strongest clinical signals are derived from non-randomized comparative studies,[3,14,15,24–26] particularly with regard to reduced fixation hardware failure rates and improved postoperative skeletal stability.

The evidence supporting the prevention of cephalometric relapse and the maintenance of occlusal stability is primarily derived from small prospective cohorts and case series.[15,24–26] Mechanistic support is provided by animal studies[3] and imaging-based investigations.[24,26] Although biologically plausible, single case reports[13,22,23] should be interpreted as hypothesis-generating rather than confirmatory evidence.

Accordingly, the clinical recommendations derived from this review should be considered adjunctive and selective rather than universally applicable.

Table 1. Characteristics of the studies included in the systematic review, evaluating the use of botulinum toxin injections into masticatory and suprahyoid muscles for occlusion management in orthognathic surgery and mandibular fracture treatment

First author	Year	Study type	Clinical scenario/ patients	Sample size (*n*)	Botulinum toxin type	Dose and dilution	Injection sites	Timing of injection	Comparator	Follow-up	Main outcomes	Relapse/ stability findings	Adverse events	Risk of bias (ROBINS-E/ case report quality)
Tukel[3]	2020	experimental controlled animal study	male New Zealand white rabbits with a unilateral unfavorable mandibular body fracture	48 animals (BTX-A group: *n* ≈ 24; control: *n* ≈ 24)	onabotulinumtoxinA (BTX-A)	10 U diluted in 0.2 mL saline per masseter muscle	bilateral masseter muscles	1 week before mandibular osteotomy and fixation	saline injection + identical fixation	21 days post-op (euthanasia and analyses at day 21)	radiodensitometry (BMD), biomechanics (failure load, bending modulus), histology/ healing score, histomorphometry (BV/TV, Tb.Dm, etc.)	significantly higher BMD, failure load, bending modulus, histological healing score, BV/TV, and Tb.Dm in the BTX-A group vs. control	no BTX-A-related adverse events reported	moderate (a controlled experimental design; an animal model limits clinical generalizability)
Akbay[13]	2014	case report	pPediatric displaced condylar fracture	1	BoNT-A	not fully specified	masseter, temporalis, medial pterygoid	after failed splint therapy	none	1 month	condylar realignment	near-complete recovery	none	high (a single case, incomplete dosing data)
Canter[14]	2007	prospective case series	adult unilateral condylar fractures treated conservatively	10	Botox® (BoNT-A)	100 IU total, 20 IU/mL	masseter, temporalis (anterior), medial and lateral pterygoid	early post-trauma	none	≥6 months	stable occlusion, maintained reduction	no relapse observed	none	moderate (no control group, a small sample)
Coclici[15]	2021	prospective imaging study	Class II orthognathic surgery	5	Dysport® (BoNT-A)	20 IU total	bilateral anterior bellies of digastric	intraoperative	none	6–9 months	ultrasound muscle morphometry	changes suggest a reduced relapse risk	none	moderate (a small sample, surrogate outcomes)
Seok[22]	2013	case report	post-traumatic anterior open bite	1	BoNT-A	≈20 IU (inferred)	anterior belly of digastric	post-revision surgery	none	several months	immediate bite closure	stable occlusion	none	high (a single case, a descriptive outcome)
Kang[23]	2019	case report	Class II hyperdivergent open bite after orthognathic surgery	1	Meditoxin® (BoNT-A)	20 IU total	anterior belly of digastric	intraoperative	none	15 months	overbite increase	no relapse at long-term follow-up	none	high (a single case, no comparator)
Mücke[24]	2016	prospective controlled study	adult Class II division 2 after mandibular advancement	32	BoNT-A	20 IU	suprahyoid muscles	preoperative	surgery alone	12 months	cephalometric stability	reduced relapse vs. control	none	moderate (non-randomized, a small BoNT group)
Shin[25]	2018	retrospective controlled study	skeletal Class III undergoing BSSRO setback	16	BoNT-A	25 IU per masseter	bilateral masseters	immediate postoperative	no injection	6 months	reduced plate fracture incidence	relapse unchanged	none	moderate-to-high (a retrospective design)
Ban[26]	2023	case series + narrative review	Class II orthognathic surgery	5	BoNT-A	≈20 IU	anterior bellies of digastric	intraoperative	none	9 months	cephalometric stability	no sagittal or vertical relapse	none	moderate (a case series, a well-described protocol)

BSSRO – bilateral sagittal split ramus osteotomy; BTX-A – botulinum toxin type A; BoNT-A – botulinum neurotoxin type A; BMD – bone mineral density; BV/TV – bone volume/tissue volume; Tb.Dm – trabecular diameter.

Mechanism of action and rationale

Botulinum toxin type A exerts its effects by cleaving SNAP-25, a component of the SNARE complex that is essential for acetylcholine release at the neuromuscular junction.[4] This inhibition results in temporary flaccid paralysis of the targeted muscles, typically lasting approx. 3–6 months. Muscle weakening begins within 2–5 days after injection, reaches its peak effect at approx. 2 weeks, and gradually resolves as axonal sprouting and neuromuscular reinnervation occur.[17]

The rationale for BTX-A use in orthognathic and trauma-related surgery lies in its ability to temporarily reduce the contractile forces exerted by muscles that may otherwise compromise the stability of the recently repositioned bones or fractured segments. This effect is particularly relevant for muscles such as the masseter, temporalis, medial pterygoid, and digastric, whose activity influences mandibular stability and position.[13–15,24,26] By attenuating these biomechanical forces, BTX-A may facilitate bony healing, reduce skeletal relapse, and optimize occlusal relationships during the early postoperative recovery phase.[3]

Applications in orthognathic surgery

Masseter muscle injections

In mandibular setback procedures, such as bilateral sagittal split ramus osteotomy (BSSRO), the masseter and medial pterygoid muscles generate posterior and superior forces that may compromise fixation stability and contribute to plate fracture or skeletal relapse. Shin et al. reported a reduction in titanium miniplate fractures following the bilateral postoperative administration of BTX-A into the masseter muscles.[25] The study demonstrated a significant difference in plate failure rates (12.5% vs. 50%), favoring the BTX-A group, suggesting that the temporary attenuation of masticatory forces may provide protection for fixation constructs during osseous consolidation.[25]

Although the BTX-A group did not demonstrate statistically significant differences in the cephalometric markers of skeletal relapse, including the measures of mandibular position relative to the cranial base (e.g., the Sella–Nasion–B-point (SNB) angle), the mandibular plane angle and the gonial angle, the reduced incidence of mechanical failure highlights the potential utility of muscle inactivation in enhancing fixation integrity.[25] These findings suggest that BTX-A may be particularly beneficial in patients with high bite forces, severe deformities requiring substantial segmental movements, or compromised fixation stability.

Digastric muscle injections

The anterior belly of the digastric muscle plays a critical role in mandibular depression. In patients undergoing counterclockwise mandibular rotation for Class II hyperdivergent malocclusion with anterior open bite, excessive or unbalanced digastric activity may generate downward forces that counteract surgical movements and predispose to relapse. Traditionally, suprahyoid myotomy has been employed to reduce these forces; however, this approach is associated with potential complications, including hemorrhage, dysphagia and prolonged recovery.

Coclici et al. used ultrasound imaging to evaluate dimensional changes in the digastric muscle following BTX-A injection, and reported statistically significant increases in muscle length, together with reductions in width and cross-sectional area, indicating the elongation and thinning of the muscle.[15] These morphological changes were consistent with reduced muscle tone, supporting the potential role of BTX-A in mitigating downward mandibular relapse.[15]

In a five-patient case series, Ban et al. demonstrated the long-term stability of cephalometric measurements and occlusion following BTX-A injection into the anterior belly of the digastric muscle at the time of bimaxillary surgery.[26] No significant differences in SNB or ANB angles were observed between early and late postoperative assessments, suggesting that a single intraoperative injection may provide sufficient functional attenuation during the critical period of skeletal relapse risk.[26]

Prevention of deep bite relapse

In Class II division 2 malocclusion with deep bite, relapse following mandibular advancement may be influenced by increased vertical forces generated by the masticatory muscles, particularly the masseter and temporalis. Mücke et al. conducted a prospective study comparing 24 control patients with 8 patients who received preoperative BTX-A injections into the masticatory muscles before mandibular advancement.[24] After 1 year of follow-up, cephalometric analysis demonstrated significantly improved postoperative stability in the BTX-A group, particularly regarding vertical overbite and mandibular plane angle measurements.[24] These findings highlight the potential contribution of muscle tone to skeletal relapse and suggest that BTX-A may represent a minimally invasive alternative to myotomy or rigid overcorrection.

Applications in facial trauma and fractures

Conservative treatment of condylar fractures

Condylar and subcondylar fractures present unique management challenges due to their proximity to the temporomandibular joint (TMJ) and associated musculature. Traditional closed reduction approaches often depend on patient compliance and physical therapy. In both pediatric and adult patients with displaced but reducible fractures, BTX-A has been used as an adjunct to reduce muscular forces and facilitate passive remodeling.

Canter et al. administered BTX-A injections into the masseter and temporalis muscles in adults with unilateral condylar fractures managed conservatively.[14] The authors reported favorable anatomical reduction and functional recovery without the need for fixation hardware.[14] Similarly, Akbay et al. described the use of BTX-A in the masseter, temporalis and medial pterygoid muscles in a pediatric patient, resulting in complete radiographic healing and restoration of occlusion.[13] These reports illustrate the potential of BTX-A to facilitate fracture healing by reducing displacing muscular forces, particularly in selected patients where compliance, age or anatomical considerations may limit conventional management approaches.

Enhancement of fracture healing

Beyond its role in mechanical stabilization, BTX-A may also influence bone metabolism. In an experimental rabbit model, Tukel et al. demonstrated that BTX-A injection into the masseter muscle enhanced bone healing following surgically created mandibular fractures.[3] Radiodensitometric, histomorphometric and biomechanical analyses showed significantly greater bone mineral density, trabecular volume and failure load in BTX-A-treated animals as compared to controls.[3] These findings suggest that BTX-A may not only reduce mechanical stress, but also favorably modulate the biological environment involved in fracture repair.

Effects on occlusion and masticatory forces

The occlusal forces generated by the masticatory muscles are a crucial factor in postoperative dynamics.[27–31] The administration of BTX-A into the masseter muscle has been shown to produce a quantifiable reduction in bite force, typically in the range of 20–40%, with effects lasting approx. 3–4 months.[4] This temporary attenuation of masticatory forces may facilitate osseous consolidation by reducing excessive mechanical loading during the early healing phase, thereby promoting occlusal settling and protecting fixation constructs.

Cephalometric and ultrasonographic studies further support these functional changes. Coclici et al. observed increased length and reduced width of the digastric muscle following BTX-A injection, consistent with decreased muscle tone.[15] These morphological changes were accompanied by improved stability of occlusal parameters, including overbite and incisal display.[15] The modulation of muscle force vectors may therefore be particularly beneficial for optimizing occlusal outcomes, reducing relapse in high-risk skeletal configurations, and minimizing parafunctional loading during the early postoperative healing phase.[32]

Dosing and injection protocol variability

Considerable heterogeneity was observed across the included studies with respect to botulinum toxin formulation, dosage, dilution protocol, and timing of administration. Most clinical studies used onabotulinumtoxinA (e.g., Botox®, Meditoxin®),[3,13,14,22–26] whereas only one study employed abobotulinumtoxinA (Dysport®).[15]

For onabotulinumtoxinA, the most commonly reported doses ranged from 25–40 units per masseter muscle,[13,14,25] 15–30 units per temporalis muscle,[13,14] 10–20 units per medial pterygoid muscle,[13,14] and 5–10 units per anterior belly of the digastric muscle,[15,22,23,26] with injections typically distributed across multiple sites within each muscle. When reported, dilution concentrations were approx. 20 IU/mL; however, the injected volume per site was inconsistently described across studies (Table 1).

AbobotulinumtoxinA was used in a limited number of studies at a total dose of 20 units for bilateral injections into the anterior bellies of the digastric muscles; however, detailed information regarding dilution protocols and injection volumes was frequently omitted. Since botulinum toxin units are formulation-specific and not biologically interchangeable, no dose conversion between onabotulinumtoxinA and abobotulinumtoxinA was applied. Consequently, comparisons between formulations were qualitative and based on the reported clinical outcomes rather than the assumed numerical equivalence.

This variability highlights the lack of standardized dosing protocols and limits direct comparisons across studies.

Contraindications and ethical considerations

The use of BTX-A as an adjunct for surgical stabilization is currently considered off-label, as regulatory approvals are limited to cosmetic[19] and selected neuromuscular indications.[33] Its application in orthognathic and maxillofacial trauma surgery therefore requires thorough informed consent, with the explicit discussion of its investigational nature in this clinical context.

Absolute contraindications include known hypersensitivity to BTX-A or human albumin, active infection at the intended injection site, neuromuscular junction disorders (e.g., myasthenia gravis), and pregnancy or lactation. Caution is also warranted in patients with coagulopathies or those receiving anticoagulant therapy.

Pediatric use remains particularly controversial. Although BTX-A has been used in children for conditions such as cerebral palsy[34] and dystonia, its application in craniofacial fractures or orthognathic surgery lacks robust evidence regarding safety and long-term outcomes. Growth-related skeletal changes may influence treatment effects,[32,35,36] rendering pediatric applications ethically sensitive. Accordingly, the use of BTX-A for orthognathic or trauma-related indications in pediatric patients cannot currently be recommended outside ethically approved research protocols.[31,37,38] In such settings, the informed consent process should explicitly address the off-label nature of the intervention, the absence of long-term data on

craniofacial growth, and the availability of the established alternative treatment options.

Repeated administration of BTX-A carries a potential risk of neutralizing antibody formation,[39] which may reduce treatment efficacy over time. Consequently, its use should be reserved for carefully selected patients at high risk of relapse or fixation-related complications until robust long-term evidence becomes available.

Economic and accessibility implications

The integration of BTX-A into surgical workflows introduces additional cost and logistical considerations:

- cost-effectiveness: A vial of onabotulinumtoxinA (100 U) typically costs €150–€300, with per-procedure requirements ranging from 50 to 100 U for bilateral injections. In comparison with the costs associated with hardware replacement, the correction of skeletal relapse, or revision surgery, this expense may be justified in selected high-risk cases[40];
- accessibility: the administration of BTX-A requires detailed anatomical knowledge and, in some settings, ultrasound guidance, potentially limiting its implementation to experienced surgeons or multidisciplinary centers with appropriate expertise;
- insurance and regulatory considerations: Reimbursement is uncommon for off-label surgical applications, potentially contributing to disparities in access across healthcare systems. The development of clinical guidelines and further cost–benefit analyses may support broader evaluation and adoption;
- workflow integration: BTX-A injection can be incorporated into the perioperative workflow without substantial additional operative time (<10 min) and with minimal patient discomfort, suggesting potential feasibility within standard surgical protocols as supporting evidence continues to evolve.

Limitations and considerations

Despite the growing body of supportive evidence, several limitations must be acknowledged. Most available studies are characterized by small sample sizes, case reports or experimental animal models, limiting the generalizability of current findings. The standardization of dosing protocols, injection techniques, and timing of administration remains a major area of clinical variability. Furthermore, repeated BTX-A administration may be associated with immunogenicity and the potential development of neutralizing antibodies, which could reduce therapeutic efficacy over time.

The principal limitation of the available literature is the predominance of low-level evidence, including case reports, small case series and experimental animal studies. Only a limited number of non-randomized comparative investigations have been published, and no randomized controlled trials (RCTs) have specifically evaluated BTX-A as an adjunctive intervention in orthognathic surgery or maxillofacial trauma.

Heterogeneity in dosing protocols, targeted muscles, outcome definitions, and follow-up duration further limits direct comparison between studies. Consequently, current evidence should be interpreted as supportive but not definitive.

The interpretation of outcomes is potentially confounded by variability in fixation systems, the magnitude of skeletal movements, postoperative rehabilitation protocols, and patient-specific factors, including baseline bite force and parafunctional activity. These variables were inconsistently reported across studies and may independently influence the risk of skeletal relapse or hardware failure, irrespective of BTX-A administration.

Based on current evidence, BTX-A may be considered in selected high-risk clinical scenarios, including patients with elevated masticatory forces, substantial mandibular movements, hyperdivergent Class II patterns, or compromised fixation stability. Beyond skeletal and occlusal outcomes, patient-reported outcomes and postoperative satisfaction represent important endpoints in orthognathic surgery and should be considered alongside objective functional measures when evaluating adjunctive interventions such as BTX-A.[41] At present, the routine use of BTX-A in standard-risk cases is not supported by sufficient evidence.

The adverse effects associated with BTX-A administration are generally uncommon, but may include transient dysphagia, unintended muscle weakness, asymmetry, or hematoma formation. Appropriate anatomical localization and ultrasound guidance may help reduce these risks. However, the potential benefits of any adjunctive intervention must be considered within the broader risk profile of orthognathic procedures, which includes established complications, such as nerve injury, infection and functional impairment, as extensively discussed in recent risk assessment analyses.[42]

Additionally, the ethical considerations surrounding BTX-A use in pediatric populations and its potential role as an alternative or adjunct to conventional fixation strategies require further investigation.

Large-scale RCTs with long-term follow-up are necessary to define the precise role of BTX-A in orthognathic and maxillofacial trauma surgery. The development of consensus-based protocols will be essential to support evidence-based adoption and ensure consistency in patient selection, treatment application and clinical outcomes.

Conclusions

The injections of BTX-A into the masticatory and suprahyoid muscles represent a biologically plausible and minimally invasive adjunctive strategy for enhancing skeletal and occlusal stability following orthognathic surgery

and facial fractures. Current evidence suggests potential benefits in reducing hardware failure rates, skeletal relapse and muscle-related complications.

Nevertheless, substantial heterogeneity in treatment protocols, the absence of regulatory approval for these specific indications, and limited long-term evidence currently constrain routine clinical adoption. Ethical implementation requires transparent communication regarding the off-label and investigational nature of BTX-A use, particularly in pediatric and other vulnerable populations.

As the understanding of musculoskeletal neuromodulation continues to advance, BTX-A may evolve from an ancillary therapy into an integrated component of personalized maxillofacial surgery, offering the potential to balance biomechanical modulation, biological healing processes and economic considerations to optimize patient outcomes.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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