Dental and Medical Problems

BIMONTHLY ISSN 1644-387X (PRINT) ISSN 2300-9020 (ONLINE) www.dmp.umw.edu.pl

2025, Vol. 62, No. 2 (March–April)

Impact Factor (IF) – 2.7
Ministry of Science and Higher Education – 70 pts

Wroclaw Medical University

Dental and Medical Problems

ISSN 1644-387X (PRINT) ISSN 2300-9020 (ONLINE) www.dmp.umw.edu.pl

Dental and Medical Problems is an international, peer-reviewed, open access journal covering all aspects of oral sciences and related fields of general medicine, published bimonthly by Wroclaw Medical University.

BIMONTHLY
2025, Vol. 62, No. 2
(March–April)

Editorial Office
Marcinkowskiego 2–6
50-368 Wrocław, Poland
Tel.: +48 71 784 12 05
E-mail: dental@umw.edu.pl

Publisher
Wroclaw Medical University
Wybrzeże L. Pasteura 1
50-367 Wrocław, Poland

Online edition is the original version of the journal

Manuscript editing
Joanna Gudarowska
Jolanta Prazeres

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Indexed in: PubMed/MEDLINE, Web of Science, Clarivate Journal Citation Report, Scopus, ICI Journals Master List, DOAJ, WorldCat, Embase, Polska Bibliografia Naukowa, EBSCO, Crossref, CLOCKSS

Typographic design: Monika Kolęda, Piotr Gil
Cover: Monika Kolęda
DTP: Adam Barg
Printing and binding: Drukarnia I-BiS Bierońscy Sp.k.

Dental
and Medical Problems

BIMONTHLY 2025, Vol. 62, No. 2 (March–April)

ISSN 1644-387X (PRINT)
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www.dmp.umw.edu.pl

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Is malocclusion a risk factor for obstructive sleep apnea and temporomandibular disorders? An orthodontic point of view

Anna Maria Paradowska-Stolarz[A–F]

Division of Facial Developmental Defects, Department of Maxillofacial Orthopedics and Orthodontics, Faculty of Dentistry, Wroclaw Medical University, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):197–199

Address for correspondence
Anna Paradowska-Stolarz
E-mail: anna.paradowska-stolarz@umw.edu.pl

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on August 3, 2024
Reviewed on August 6, 2024
Accepted on October 7, 2024

Published online on April 18, 2025

Cite as
Paradowska-Stolarz AM. Is malocclusion a risk factor for obstructive sleep apnea and temporomandibular disorders? An orthodontic point of view. *Dent Med Probl.* 2025;62(2):197–199. doi:10.17219/dmp/194232

DOI
10.17219/dmp/194232

Keywords: orthodontics, obstructive sleep apnea, temporomandibular disorders, malocclusion

There is a strong connection between malocclusion and obstructive sleep apnea (OSA).

In recent years, obstructive sleep apnea (OSA) has become a challenging medical problem. More and more patients are diagnosed with this disorder, and the awareness of OSA is increasing among physicians as well as dentists. Obstructive sleep apnea is a disorder in which, during sleep, muscles relax so much that they can block the airways, so by definition this is a muscular condition. Since the dentist is a frequently visited healthcare provider, often, it is them who can screen the patient for the diagnosis of OSA.

Some recent studies indicate that the phenomenon could be strongly linked with temporomandibular disorders (TMD), which are also related to muscular tension, with bruxism being the manifestation of such disorders; the coincidence of those conditions is well known in today's medical world.[1–3] Temporomandibular disorders comprise the disorders of temporomandibular joints (TMJs), the masticatory muscles and the surrounding structures.[4,5] The reduced sleep time and the low quality of sleep increase the risk of TMD and other disorders.[2] Those disorders might be related to many general medical conditions, i.a., many systemic diseases, like hypertension, hyperinsulinism and rheumatoid arthritis.[1–5] Temporomandibular disorders also strongly affect sleep, lowering its quality. They might be related to the occurrence of OSA as well. Impaired sleep quality and insufficient oxygenation during sleep lead to the worsening of the psychosocial condition of individuals, causing general health problems and hindering the patient's well-being.[6] On the other hand, the psychosocial background of TMD is undeniable, as stress is one of the most common factors influencing TMJs and the orofacial muscles, as mentioned in the literature.[4,7] Also, the presence of oral behaviors, such as sleep bruxism, causes hypoxia in the patient, leading to sleep-related breathing disorders (SRBD) of various intensity.[8] Yet, this relationship needs further investigation, as the definition of bruxism has changed; it is no longer considered 'parafunctional behavior', and instead, 2 conditions are differentiated – sleep and awake bruxism.[9] The orofacial complex could be compared to a structure of communicating vessels, in which one element strongly influences another. Therefore, researchers should investigate any connections between those elements.

Recently, there has been a strong dispute on whether or not TMD are connected with malocclusion in any way, and if orthodontic treatment influences TMJs. In some studies, TMD are reported as the 2nd most common (after apical root resorption) complication of orthodontic treatment,[10,11] though the connection between TMD and malocclusion, as well as orthodontic treatment, is highly disputable and not quite clear.[12] Some researchers found a strong relationship between malocclusion and TMD,[13] while others deny the existence of it.[14] The connection is inexplicit, although in the maxillofacial and orofacial regions, the parts of the stomatognathic system obviously impact each other. It is evident, though, that TMD have a multifactorial background and the definition of their origin is challenging.[1–5] Today, we are witnessing a swing of interest with regard to TMD; the crucial issue seems to be determining the cause of those problems, as this might be the key to establishing successful treatment methods for patients in the future.

As mentioned above, TMD may co-occur with OSA. The main cause of OSA is the narrowing of the airways. However, the new guidelines suggest avoiding surgical treatment in OSA patients, as it may cause considerable discomfort.[15]

It is a well-known fact that orthodontic treatment may affect the dimensions of the airways.[16] Also, the coincidence of malocclusion and OSA remains a huge problem.[17] Both maxillary and mandibular advancement enlarges the dimensions of the upper airways. In Class II malocclusion, the functional advancement of the mandible, generating its forward movements, increase the airway volume measured at the 3rd cervical vertebra (C3).[16] On the other hand, the traction of the maxilla in Class III malocclusion also increases the airway volume.[17] Furthermore, it has been shown that OSA patients present with narrower maxillae. In children, the potential treatment can be guided by maxillary expansion with both removable and (preferably) fixed appliances, while in adults, surgically assisted maxillary expansion is required.[18] Also, while in children it is possible to use functional treatment for Class II patients and maxillary protraction for Class III patients, in adults, the problem becomes more complex. In many of those patients, the only chance for stable orthodontic treatment is orthognathic surgery, especially according to face-driven orthodontics, which is a current treatment trend. The trend is also known as 'facially driven orthodontics', and consists in preserving or enhancing facial features, while improving the functional aspects, i.e., ensuring the correct contact of the teeth, wide airways and correct muscular tension. This is a shift from the "only occlusion matters" point of view to a holistic approach in orthodontics.[19] Another issue is adenotonsillectomy, considered by some researchers as a predictor of OSA or an indication for treating OSA.[17] In the patients requiring orthognathic surgery, the improper growth pattern may have a genetic background, yet the hindered airflow in the airways could cause changes in the person's posture, leading to muscular function disorders and being a risk factor for malocclusion in the skeletal pattern. The question is how far could the surgical approach regarding the correction of malocclusion be modified, and would it influence the dimensions of the airways that much?

What is also problematic is the definition of the upper airways and the diagnosis of OSA. The measurement of the upper airway volume could refer to some other point, and not the height of the C3, as mentioned in the literature.[16] The American Association of Orthodontists (AAO) published guidelines, according to which it is not the orthodontist who should diagnose severe breathing problems; the patient should be referred to a general practitioner, who makes diagnosis.[20] Most of the published research concerning orthodontic treatment, as well as OSA, is initiated by dentists, including orthodontists, without the participation of general doctors, which could be a source of bias, with the possibly subjective conclusions drawn by orthodontists or orthognathic surgeons alone. Nevertheless, in the AAO White Paper, it is clearly stated that the orthodontist should be involved in the process of screening patients for OSA,[20] as there might be a strong connection between orthodontic treatment needs and sleeping disorders, including sleep apnea.[21] Even though the presented studies account for the important role of orthodontic treatment (especially when associated with orthopedic improvement, with either functional treatment or surgical advancement), the doubts still remain. The problem is that even if there is significant improvement in the airway volume, the real impact on health remains disputable. Although the airway volume measured by means of cone-beam computed tomography (CBCT) seems to be enhanced, the measured blood parameters may not confirm such improvement.[21] Therefore, more studies based on polysomnography (PSG) and blood tests should be undertaken to confirm or reject the the effect of orthodontic treatment. Additionally, separate studies should be conducted with regard to functional treatment, palatal expansion and surgically associated orthodontics. This could be an interesting pathway for investigation in the nearest future. As a summary, Fig. 1 collects all the most common causes of OSA.

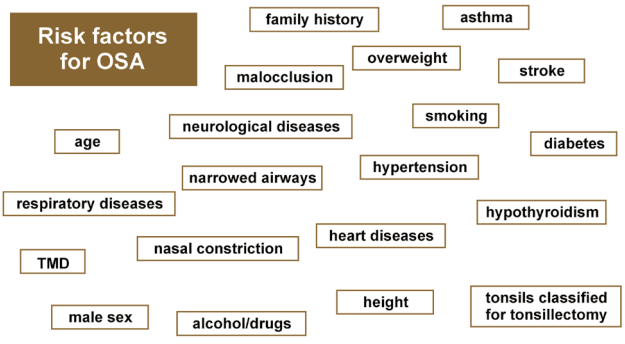

Fig. 1. Most common risk factors for obstructive sleep apnea (OSA)
TMD – temporomandibular disorders.

To conclude, it is worth emphasizing that OSA should remain in the circle of physicians' interest. The diagnostic and treatment procedures for OSA should be based on the guidelines of the American Academy of Sleep Medicine (AASM).[22] Those are thought to be the most important guidelines for preventing, diagnosing and treating OSA. Investigating this condition may lead to interesting conclusions and change the perception of the problem. The questions still remain unanswered – What was first – OSA or malocclusion? Could we orthodontically modify the growth of children to avoid severe breathing problems? Is malocclusion a crucial risk factor for severe breathing problems?

ORCID iDs

Anna Maria Paradowska-Stolarz https://orcid.org/0000-0003-2817-1445

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Indications for antibiotic prophylaxis, and algorithms for dental management after open and endovascular surgery in patients with aortic diseases

Dorota Łyko-Morawska[1,A–F], Michał Serafin[2,A–D], Łukasz Szkółka[1,A–D], Maryam Kazelka[3,A–C], Millena Levin[3,A,B], Emila Senderek[2,A–C], Agnieszka Święszek[1,A–C], Mariusz Szuta[4,E,F], Wacław Kuczmik[1,E,F]

[1] Department of General Surgery, Vascular Surgery, Angiology and Phlebology, Faculty of Medical Sciences in Katowice, Medical University of Silesia in Katowice, Poland
[2] Student Scientific Society, Department of General Surgery, Vascular Surgery, Angiology and Phlebology, Faculty of Medical Sciences in Katowice, Medical University of Silesia in Katowice, Poland
[3] University of South Florida (USF) Health Morsani College of Medicine, Tampa, USA
[4] Department of Oral Surgery, Jagiellonian University Medical College, Krakow, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):201–207

Address for correspondence
Dorota Łyko-Morawska
E-mail: dorota.lyko@sum.edu.pl
Michał Serafin
E-mail: michal.j.serafin@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on July 25, 2024
Reviewed on August 27, 2024
Accepted on September 17, 2024

Published online on April 30, 2025

Cite as
Łyko-Morawska D, Serafin M, Szkółka Ł. Indications for antibiotic prophylaxis, and algorithms for dental management after open and endovascular surgery in patients with aortic diseases. *Dent Med Probl.* 2025;62(2):201–207. doi:10.17219/dmp/193485

DOI
10.17219/dmp/193485

Keywords: stent-graft, dentistry, recommendations, VGEIs, antibiotic prophylaxis

As the population of patients with aortic prostheses continues to grow, it becomes increasingly vital to implement comprehensive and rigorous protocols for antibiotic prophylaxis during dental procedures. That ensures strict adherence to the updated guidelines in order to effectively prevent the risk of life-threatening infections associated with vascular grafts and stent-grafts, thus safeguarding patient outcomes over the long term.

Introduction

Cardiovascular diseases (CVD) are the leading cause of death worldwide, including the rising incidence of aortic aneurysms.[1,2] An aortic aneurysm is defined as a dilation of the aortic cross-section by more than 50% of its normal diameter. Abdominal aortic aneurysms (AAAs) affect 2–8% of patients in developed countries and are mostly asymptomatic, with a high mortality rate of approx. 80% if the aneurysm ruptures.[3]

Recent advancement in radiological preventive examinations (e.g., the Healthy Aorta Program in Poland), along with the development of surgical techniques, material science and endovascular procedures, have led to an increasing number of patients reporting to vascular departments for treatment.[4,5] The two primary surgical approaches for treating AAAs are open aneurysm repair and endovascular aneurysm repair (EVAR). Both procedures involve the insertion of alloplastic material intended to restore or protect the pathologically altered segment of the aorta. Significant complications of such treatment are vascular graft or endograft infections (VGEIs), which can result from transient bacteremia related to various medical procedures, including dental procedures.[6–10] Therefore, preoperative and postoperative dental consultations are essential to identify and eliminate potential odontogenic

sources of infection. Unfortunately, there is limited data regarding antibiotic prophylaxis in patients with vascular grafts or endografts.

The present study aims to provide practical recommendations for the management and prophylaxis of infections in patients with vascular prostheses, with a focus on dental care.

Methods

Purpose of the guidelines

Current European and American guidelines lack specific recommendations for antibiotic prophylaxis during dental procedures for patients with vascular prostheses. The existing preventive strategies are primarily based on guidelines for infectious endocarditis. To fill this gap, a multidisciplinary team of vascular surgeons, maxillofacial surgeons and dentists developed these guidelines to assist healthcare professionals in preventing infections in this high-risk group. The guidelines target dentists, maxillofacial surgeons and other providers involved in the care of patients with vascular grafts, including those with grafts in the supra-aortic trunks, thoracic or abdominal aorta. They aim to ensure effective prophylaxis against VGEIs during and after dental procedures.

Authors

These guidelines have been developed by a multidisciplinary team composed of experts in vascular surgery, maxillofacial surgery and dentistry. The collaborative effort ensures that the recommendations reflect a comprehensive and specialized approach to antibiotic prophylaxis in dental settings for patients with vascular prostheses, integrating expertise from multiple fields to address this specific clinical need.

Literature selection

The literature search for these guidelines utilized PubMed and Scopus, covering the period from January 2000 to December 2023. Studies were selected based on their peer-review status and relevance, following a hierarchy of evidence: systematic reviews and meta-analyses were prioritized, followed by randomized controlled trials (RCTs), observational studies and expert opinions.

Study protocol

These recommendations were prepared using the international Appraisal of Guidelines for Research and Evaluation (AGREE) Reporting Checklist (supplementary material, available from the corresponding author on reasonable request).

Recommendations

Management of the patient qualified for vascular treatment due to an aortic aneurysm

Before undergoing vascular treatment, it is essential for the patient to have a dental consultation that includes both a clinical examination and radiological diagnostics, such as a pantomographic X-ray or cone-beam computed tomography (CBCT). The patient must obtain a statement from a dentist, confirming the absence of odontogenic foci. It is important to emphasize that a clinical dental examination alone is not sufficient for a complete assessment; a thorough radiological evaluation is necessary for surgical qualification.

Management of the patient after aortic surgery

According to the guidelines of the European Society for Vascular Surgery (ESVS),[10] and the Society for Vascular Surgery,[11] antibiotic prophylaxis in the dental office is currently recommended after both open repair surgery with alloplastic vascular prostheses and EVAR with a stent-graft. It is also recommended before any dental procedures involving the manipulation of the gingival or apical regions of the teeth, or the perforation of the oral mucosa, including scaling and root planing, and endodontic treatment.[10,11]

The types of dental procedures with recommendations for antibiotic therapy in patients after vascular procedures are shown in Table 1. However, in the aforementioned guidelines, there are statements where antibiotic prophylaxis is required for certain dental procedures, but no information is provided about the type and exact dosage of antibiotics. Additionally, these guidelines are based on the American College of Cardiology/American Heart Association (ACC/AHA) guidelines for patients with endocarditis.[12]

Based on the previously published guidelines,[10–13] recent literature on infectious endocarditis, VGEIs and oral bacterial flora,[14–17] and clinical experience, the authors of this article have prepared recommendations regarding antibiotic prophylaxis for patients with vascular prostheses (Tables 2 and 3).

Discussion

With over 35 million pulsatile movements per year, the aorta presents a challenging environment for the implantation of vascular prostheses and stent-grafts, which must endure over time without complications, such as endoleaks, false aneurysms, or one of the most challenging

Table 1. Recommendations for antibiotic therapy in dental procedures in patients after the implantation of alloplastic material

Type of procedure	Diagnosis	Antibiotic prophylaxis	Dental procedures
Endovascular procedures	status after the endovascular treatment of an aortic aneurysm with a stent-graft	required	– root canal treatment – peripheral surgery (the resection of the tooth tips) – tooth extraction – implant placement – periodontal surgery (including flap techniques, and supra-guminal and sub-guminal curettage) – scaling and root planing*
Hybrid procedures	status after the treatment of the aorta with endovascular techniques and vascular prosthesis implantation	required	
	status after the treatment of the aorta, using an endovascular prosthesis and an extra-anatomical alloplastic bypass	required	
Open surgery procedures	open surgical treatment with an alloplastic prosthesis	required	
	status after any surgical procedures within the previously infected vascular prosthesis	required ** (not only antibiotic prophylaxis, but also postoperative antibiotic therapy)	

* The guidelines do not provide detailed information on supra-guminal and sub-guminal scaling; in this case, the possibility of interrupting the continuity of the mucous membrane is important.
** In the available literature, there is no specific data regarding patients who underwent interventions within the previously infected vascular prostheses. It is generally assumed that these patients are classified within the broader group of individuals with implanted vascular prostheses.

Table 2. List of dental procedures with the algorithms of the perioperative procedure and antibiotic therapy

Dental procedure	Antibiotic prophylaxis	Recommended additional procedures	Comments
Conservative treatment of the teeth	not recommended	–	–
Infiltrative/conduction anesthesia	not recommended	–	in non-infected tissues
	recommended	–	in infected tissues
Endodontic treatment	recommended	– dental dam – rinsing protocol – sealed temporary fillings	–
Periapical surgery	recommended	–	–
Scaling and root planing	recommended	–	–
Tooth extraction	recommended	–	–
Implant placement	recommended	–	there is no literature data on the safety of implant therapy in this patient population

Table 3. Antibiotic type and dosage in dental procedures with the recommended prophylaxis in patients with vascular prostheses

Patient group	Timing of prophylaxis	No allergy to penicillin		Allergy to penicillin	
		antibiotic	dosage	antibiotic	dosage
Adults	single dose 30–60 min before the procedure	amoxicillin	2 g p.o.	cephalexin	2 g p.o.
		ampicillin	2 g i.m. or i.v.	azithromycin or clarithromycin	500 mg p.o.
		cefazolin or ceftriaxone	1 g i.m. or i.v.	doxycycline	100 mg p.o.
		–	–	cefazolin or ceftriaxone	1 g i.m. or i.v.
Children		amoxicillin	50 mg/kg p.o.	cephalexin	50 mg/kg p.o.
		ampicillin	50 mg/kg i.v. or i.m.	azithromycin or clarithromycin	15 mg/kg p.o.
		cefazolin or ceftriaxone	50 mg/kg i.v. or i.m.	doxycycline	<45 kg: 2.2 mg/kg p.o. >45 kg: 100 mg p.o.
		–	–	cefazolin or ceftriaxone	50 mg/kg i.v. or i.m.

p.o. – per os; i.v. –intravenously; i.m. – intramuscularly.
Cephalosporins should not be used in an individual with a history of anaphylaxis, angioedema or urticaria related to the use of penicillin.

complications, VGEIs. As the number of patients treated with aortic prostheses or stent-grafts continues to rise, particularly in an aging population, it is crucial to develop management algorithms for this patient group, especially in the dental office, to eliminate potential sources of infection.[18]

The etiology of prosthetic infections, including those caused by bacteria of dental origin, is complex and closely related to the unique microenvironment where the prostheses are implanted, namely the aorta and the aortic aneurysm itself. The aorta, being a large-diameter artery with wave-like variations in blood flow velocity, creates

challenging conditions for graft implantation. The material of the endograft is subjected to tension stress and constant pulsatile movements, which can cause micromovements. These micromovements may lead to the displacement of the stent-graft, resulting in blood leakage between the stent-graft and the vessel wall, or even the rupture of the endograft. Consequently, a reservoir of extravasated blood can form along the outer wall of the prosthesis/graft, which may serve as a breeding ground for bacteria, leading to the persistent superinfection of the vascular prosthesis.[19]

Abnormal epithelialization can lead to the exposure of alloplastic material within the aortic lumen, thereby increasing the risk of infection. Abnormalities in epithelialization within the graft are confirmed through the formation of a very thin layer of neointima and/or the segmental exposure of graft components, such as the wire elements of the crown, abutments and fragments of the covering material. The exposed covering material of the prosthesis and the stent-graft provides a potential site for bacterial accumulation.[18]

In the examinations of the explanted prostheses and stent-grafts, the bacterial contamination of the prosthesis wall was confirmed, particularly on the external side (aneurysm sac side). For the prostheses made of polytetrafluoroethylene (PTFE) and polyethylene terephthalate, bacterial presence was found in 84.3% and 94.7% of cases, respectively, as determined by electron microscopy.[18]

Recent studies indicate that up to 58% of VGEIs are attributed to Gram-positive bacteria, including *Staphylococcus aureus*, enterococci and coagulase-negative staphylococci. In the oral cavity, Gram-positive bacteria, predominantly streptococci, are most common. Therefore, β-lactams, including penicillins and cephalosporins, as well as macrolides, are recommended for the prevention of VGEIs. β-lactams should be considered the first-line treatment due to their efficacy against the predominant bacterial pathogens involved. Additionally, a single administration of antibiotics for prophylaxis provides coverage for approx. 4 h.[10,20–25]

The radiological signs of prosthetic infection include the presence of fluid around the vascular prosthesis and gas bubbles in the tissue adjacent to the prosthesis or the aortic aneurysm sac in the case of EVAR, particularly when contamination involves anaerobic bacterial flora.[26]

During a single procedure, the removal of the vascular prosthesis typically requires reconstruction with an extra-anatomical bypass or the use of the previously contaminated environment to restore blood flow distal to the operated area. This necessity increases the risk of subsequent infection. Additionally, patients initially deemed suitable for endovascular surgery often cannot be considered for open vascular prosthesis replacement due to their overall condition, comorbidities or medications.[27] As a result, the only remaining therapeutic options are chronic antibiotic therapy and the drainage of the periprosthetic abscess. However, such treatment may have many adverse side effects and is often ineffective. Therefore, the importance of eliminating any risk factors for vascular prosthesis infection cannot be overstated.

Dental procedures are associated with transient bacteremia. The guidelines from vascular surgery societies for managing aortic prostheses and stent-grafts emphasize the need for antibiotic prophylaxis in all dental procedures that involve the disruption of the oral mucosa and periapical surgery, including endodontic treatment, as well as scaling and root planing.

It is important to note that current vascular surgery guidelines do not provide specific recommendations regarding the optimal duration of antibiotic prophylaxis, nor do they explicitly address whether prophylaxis should be continued until the full epithelialization of the vascular prosthesis is achieved. This uncertainty may be partly attributed to the previously mentioned abnormal and often incomplete epithelialization of vascular grafts. In contrast, cardiological guidelines recommend a prophylactic period of 6 months. This discrepancy highlights a key difference between vascular and cardiological guidelines, owing to the previously discussed epithelialization issues associated with aortic prostheses.[18]

The European and American guidelines do not specify the exact doses of antibiotics to be used for dental prophylaxis.[10,11,28] They only suggest the use of prophylaxis (recommendation level 1B), referencing practices from infectious endocarditis. The authors of this article based their recommendations on the general vascular surgery guidelines for antibiotic prophylaxis in dentistry related to infectious endocarditis, as well as on the Polish recommendations regarding antibiotic groups and the recommended doses from the National Antibiotic Program.[13] However, given recent publications questioning the necessity of antibiotic prophylaxis for dental procedures in the context of infectious endocarditis,[29] it is crucial to continually monitor this topic and update guidelines in accordance with the latest evidence.

There is no contraindication to conservative dental treatment in patients with implanted prostheses or stent-grafts, provided that the oral mucosa is not compromised. The guidelines do not recommend antibiotic prophylaxis for the local anesthesia of non-infected tissues.[30]

Endodontic treatment is essential for patients with vascular prostheses or stent-grafts, as these patients are at increased risk of infection due to potential bacteremia during procedures. Vascular surgery guidelines stress the importance of antibiotic prophylaxis to mitigate this risk, which arises from bacteria potentially entering the periapical area during canal preparation, whether manual or mechanical, and through the irrigating solutions or sealing materials extending beyond the apex.[31] To reduce the risk of infection, endodontic treatment should adhere to rigorous protocols. Using a rubber dam is crucial for maintaining a sterile environment, and efforts should be

made to complete the treatment in a single session to limit exposure to pathogens. Additionally, the radiological assessment of the endodontically treated teeth is necessary to ensure treatment success and monitor for any complications.[31]

While endodontic treatment is generally preferred over extraction, the long-term prognosis of the tooth must be carefully evaluated. Teeth with extensive periapical lesions or questionable restorability are at high risk of becoming sources of chronic infection. If the long-term survival of the tooth is uncertain, its potential to harbor infection should be weighed, and the patient must be informed of the risk. The choice between endodontic treatment and extraction should be based on a comprehensive assessment of the condition of the tooth and the likelihood of a successful, infection-free outcome. If extraction is necessary, antibiotic prophylaxis is strongly recommended to prevent the introduction of pathogens into the bloodstream during the procedure.[31]

The decisions regarding modifications to antiplatelet or anticoagulant therapy must be made in close collaboration with a vascular surgeon to avoid compromising systemic health and increasing the risk of postoperative complications. Tokarek et al. noted a concerning trend where dentists independently modify or discontinue these therapies without proper consultations, potentially leading to adverse outcomes.[32] Therefore, it is essential that dentists adhere strictly to the established guidelines and work closely with the patient's medical team to ensure the best possible treatment outcomes and minimize the risk of complications.[33]

Patients with branched stent-grafts require special consideration. This innovative endovascular procedure involves reconstructing the aorta and its branches, necessitating the implantation of multiple vascular prostheses. Due to the novel nature of this procedure and the absence of specific guidelines for this patient group, extra caution is required during dental procedures. It is crucial to address any potential inflammatory foci that may arise after the implantation of a branched stent-graft, as infection could complicate the situation significantly, given the difficulty of replacing or removing the implanted prostheses.

Currently, researchers recommend antibiotic prophylaxis for all dental procedures that involve the disruption of the oral mucosa. If odontogenic inflammation is present, the source of infection should be addressed and antibiotic therapy should be extended by 3–5 days based on the patient's clinical condition. Additionally, close postoperative monitoring is essential, as potential sources of infection pose a direct risk of prosthesis infection, sepsis, and potentially, death.

In the opinion of the authors, prolonged postoperative antibiotic therapy is indicated in cases of complicated peritoneal inflammation, such as those with concomitant purulent exudation, massive inflammatory infiltration, peritoneal abscess, or the infiltration of the surrounding soft tissues. This approach aims to limit the existing bacteremia and prevent the bacterial contamination of the vascular prosthesis; however, further studies are required to validate its effectiveness.

Qualification for implant procedures in patients with implanted vascular prostheses or stent-grafts should be always carefully considered by a dentist. Patients need to be informed about potential complications, including local inflammation immediately after surgery and the development of chronic inflammation in the implant area. While there are no available studies specifically addressing peri-implantitis in this patient group, various therapeutic options should be presented to the patient, including both fixed and removable prostheses. Currently, there are no clear guidelines for the treatment of this patient group, and this type of procedure is not specifically addressed within the guidelines of vascular surgery societies.

Patients who have been diagnosed and treated for a vascular prosthesis infection should be considered at particularly high risk.

While hygienization in the dental office is typically performed by qualified hygienists, eligibility for such procedures should be assessed by a dentist. This is crucial for this patient group. Scaling and root planing, in particular, is an indication for antibiotic prophylaxis, and this requirement must not be overlooked during patient preparation.[34]

Nonetheless, an article by Özdemir Kabalak et al. underscores the fact that despite the realized need for antibiotic prophylaxis, barriers such as inconsistent adherence to guidelines, the lack of education and varying practices in dental settings continue to impede rational antibiotic use.[34] The effective management of these issues requires a standardized approach to prophylaxis and improved adherence to guidelines to minimize the risk of antibiotic resistance and optimize patient outcomes. Thus, the continuous monitoring and updating of guidelines in line with recent literature are essential to address the evolving challenges in dental care for patients with aortic prostheses and stent-grafts.

Strengths and limitations of the guidelines

The guidelines are strengthened by a comprehensive literature review from January 2000 to December 2023 across multiple reputable databases, ensuring an up-to-date evidence base. A hierarchical approach prioritizing systematic reviews, meta-analyses and RCTs adds reliability, while a multidisciplinary team brings diverse expertise to the recommendations. However, limitations include limited specific data on antibiotic prophylaxis for patients with vascular grafts or endografts in dental settings, leading to reliance on broader guidelines. Additionally, while the guidelines are built on the existing strategies for

infectious endocarditis, they may not fully address the unique needs of patients with vascular prostheses.

Conclusions

In conclusion, as the number of patients with vascular diseases, including aortic conditions, continues to grow, it is crucial to address the evolving needs of this population. Increased patient awareness regarding oral health will likely lead to a higher demand for dental procedures. Ensuring that these procedures are performed safely and in accordance with current standards is essential to prevent complications and maintain optimal outcomes. The guidelines provided emphasize the importance of tailored antibiotic prophylaxis, and highlight the need for ongoing research and updates to refine these strategies. Adhering to these recommendations will help manage the risk of infection effectively and enhance patient care in this high-risk group.

Potential resource implications

Implementing these comprehensive recommendations may increase demands for healthcare resources, as the emphasis on preoperative and postoperative dental consultations could lead to additional costs and time commitments, requiring better coordination between dental and vascular care teams. Enhanced antibiotic prophylaxis protocols might raise procedure costs and necessitate ongoing monitoring. Additionally, healthcare providers may need further training, potentially involving workshops or updated materials. While these preventive measures could reduce infections and improve outcomes, they may also impact overall healthcare resource utilization and costs. Integrating these guidelines into clinical workflows may require administrative adjustments and new protocols, making effective planning crucial to addressing these resource implications while aiming to enhance patient outcomes and prevent complications.

Update of the guidelines

To ensure the continuity and relevance of these guidelines, the authors emphasize the need for their inclusion in the forthcoming European as well as Polish recommendations for dental prophylaxis and antibiotic therapy.

ORCID iDs

Dorota Łyko-Morawska https://orcid.org/0000-0001-5812-5090
Michał Serafin https://orcid.org/0009-0006-5154-2901
Łukasz Szkółka https://orcid.org/0009-0000-3503-8074
Maryam Kazelka https://orcid.org/0009-0000-2222-7781
Millena Levin https://orcid.org/0009-0008-2716-5514
Emila Senderek https://orcid.org/0009-0002-6376-4481
Agnieszka Święszek https://orcid.org/0000-0002-5965-5891
Mariusz Szuta https://orcid.org/0000-0002-7182-4811
Wacław Kuczmik https://orcid.org/0000-0002-0927-0977

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Original paper

Effect of tablets containing a paraprobiotic strain and the cranberry extract on caries incidence in preschool children: A randomized controlled trial

Dorota Olczak-Kowalczyk[1,A–F], Anna Turska-Szybka[1,A–F], Svante Twetman[2,C–F], Dariusz Gozdowski[3,C,D,F], Paula Piekoszewska-Ziętek[1,B–F], Joanna Góra[1,B,D–F], Marta Wróblewska[4,B,C,E,F]

[1] Department of Pediatric Dentistry, Faculty of Dentistry, Medical University of Warsaw, Poland
[2] Department of Odontology, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark
[3] Department of Experimental Statistics and Bioinformatics, Warsaw University of Life Sciences, Poland
[4] Department of Dental Microbiology, Faculty of Dentistry, Medical University of Warsaw, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):209–215

Address for correspondence
Anna Turska-Szybka
E-mail: anna.turska-szybka@wum.edu.pl; aturskaszybka@gmail.com

Funding sources
The test and placebo tablets were provided free of charge by the manufacturer, and the authors' institutions funded the project.

Conflict of interest
None declared

Acknowledgements
The authors wish to thank all children and their parents for taking part in this project.

Received on August 1, 2023
Reviewed on August 24, 2023
Accepted on September 5, 2023

Published online on July 18, 2024

Cite as
Olczak-Kowalczyk D, Turska-Szybka A, Twetman S, et al. Effect of tablets containing a paraprobiotic strain and the cranberry extract on caries incidence in preschool children: A randomized controlled trial. *Dent Med Probl.* 2025;62(2):209–215. doi:10.17219/dmp/171895

DOI
10.17219/dmp/171895

Abstract

Background. Pre- and probiotics may help restore a dysbiotic oral ecosystem. The first years of life provide a window of opportunity to modulate the composition of the oral microbiota and prevent disease.

Objectives. The aim of the present study was to investigate the effect of a tablet containing inactivated *Ligilactobacillus salivarius* CECT 5317 and the cranberry extract on the development of caries in caries-active preschool children.

Material and methods. The study employed a randomized, placebo-controlled, double-blind design. Preschool children ($N = 73$) with at least one active carious lesion were enrolled and randomly assigned to the test group or the placebo group. The intervention period was 3 months. Caries was assessed according to the International Caries Detection and Assessment System (ICDAS) II criteria at baseline and after 9 months, and oral hygiene was evaluated with the simplified oral hygiene index (OHI-S). The salivary counts of *Streptococcus mutans* and *Lactobacillus* spp. were determined at baseline, and then after 3 and 9 months through the conventional cultivation on TYCSB and MRS agar, respectively.

Results. Sixty children completed the trial (a dropout rate of 19%). The baseline caries prevalence was high in both groups (~71%) and there were no major differences between the groups with regard to background variables. The 9-month incidence of initial carious lesions (ICDAS 1+2) was significantly lower in the test group as compared to the placebo group ($p < 0.05$). The plaque levels, and the salivary counts of *S. mutans* and *Lactobacillus* spp. remained unchanged in both groups throughout the study.

Conclusions. A daily intake of a tablet containing a paraprobiotic and the cranberry extract reduced the 9-month incidence of initial non-cavitated carious lesions in caries-active preschool children. The present study is one of the first to show the impact of synbiotics on the development of caries in children.

Keywords: probiotics, prebiotics, *Streptococcus*, *Lactobacillus*, *Vaccinium macrocarpon*

Introduction

Early childhood caries (ECC), defined as the presence of one or more decayed (non-cavitated or cavitated), missing (as a result of caries) or filled tooth surfaces in any primary tooth in a child aged 71 months or younger, is a global health problem associated with impaired quality of life.[1] Although ECC is largely preventable, its prevalence remains high in many European countries; for example, in Poland, nearly 80% of 5-year-olds are affected.[2] Dental caries is a non-communicable disease (NCD), sharing biological, behavioral and socioeconomic risk factors with other NCDs.[3,4] The main preventive strategies against ECC include regular tooth brushing, restricted intake of free sugars, and daily exposure to fluorides.[1] Emerging adjunct technologies may also prove useful. The pivotal role of microbial dysbiosis in the caries process suggests that pre- and probiotics could complement efforts to restore the normal balance of dental biofilm. The early years of life present a window of opportunity for modulating the oral microbiota through such interventions.[5] Recent systematic and comprehensive reviews have concluded that lactobacilli-derived probiotics, defined as live bacteria that confer a health benefit to the host, can prevent ECC when administered daily in milk or tablets.[6,7] Paraprobiotics, or inactivated probiotics, are non-viable microbial cells (intact or broken) or crude cell extracts that, when administered orally or topically in adequate amounts, confer benefits to human or animal consumers.[8]

Research on the impact of paraprobiotics in the oral cavity is limited, but previous findings suggest that inactivated strains of *Ligilactobacillus salivarius*, isolated from human breast milk, may inhibit biofilm formation.[9,10] Furthermore, a recent short-term trial using chewing tablets containing thermally inactivated *L. salivarius* HM6 showed a reduced incidence of ECC in comparison with standard treatment.[11] Natural polyphenol-containing agents are also interesting biofilm modulators with prebiotic action. For example, natural cranberries may show anti-caries properties by altering the bacterial shape and modifying dental biofilm colonization.[12–14] Interestingly, a combination of paraprobiotics and the cranberry extract may have synergistic effects, warranting further evaluation.[15] Therefore, the aim of this study was to investigate the effect of a tablet containing inactivated probiotic lactobacilli and the cranberry extract on the development of caries in preschool children with active caries. The primary outcome was caries incidence over a 9-month period, with secondary endpoints including plaque accumulation and the salivary counts of *Streptococcus mutans* and *Lactobacillus* spp. For the primary endpoint, the null hypothesis was that caries incidence would not differ between the active intervention and placebo groups.

Material and methods

Study design

The study used a randomized, placebo-controlled, double-blind design with 2 parallel arms. The intervention lasted for 3 months, and clinical and microbial examinations were conducted at baseline, and after 3 and 9 months. The project received ethical approval from the Bioethics Committee of the Medical University of Warsaw, Poland (No. KB/232/2016), and was registered at ClinicalTrials.gov (NCT 03919838).

The test and placebo tablets were provided free of charge by the manufacturer, and the project was funded by the authors' institutions.

Participants

We invited 80 systemically healthy children aged between 3 and 6 years to participate. All participants were outpatients at the Department of Pediatric Dentistry of the Medical University of Warsaw, Poland. The inclusion criteria were as follows: the presence of dental caries (at least one initial or cavitated carious lesion, recent restorations, or missing teeth due to caries (dmft ≥ 1)); the absence of oral inflammatory conditions; and no exposure to antibiotics, probiotics or professional fluoride varnishes within 1 month prior to enrollment. Exclusion criteria were chronic systemic diseases, congenital conditions (e.g., cerebral palsy, clefts, Down syndrome), ongoing medication, family relocation plans, and poor cooperation. Children with hypomineralized second primary molars were also excluded. Written informed consent was obtained from all parents/legal guardians. A review board within the Bioethics Committee of the Medical University of Warsaw monitored the allocation concealment to safeguard the children's rights throughout the project. We enrolled the children consecutively based on a sample size calculation, in which α (the probability of a type-I error) was set at 0.05 and β (the probability of a type-II error) was 0.20. In order to detect a 50% difference in the development of caries between the groups (the anticipated mean caries incidence 2.0 vs. 1.0), 70 children (35 in each group) were required. Therefore, enrollment was extended to 80 children to allow for potential dropouts. A flowchart is presented in Fig. 1.

Randomization

We randomly assigned eligible children into the test group or the placebo group with the aid of software that used permuted blocks of uniform size (three), containing computer-generated numbers. The random number was enclosed in an opaque envelope provided to the examiner before the baseline examination. All people involved (children/parents, clinicians/examiners, research group) remained fully blinded throughout the trial, and an independent monitor guaranteed the allocation concealment.

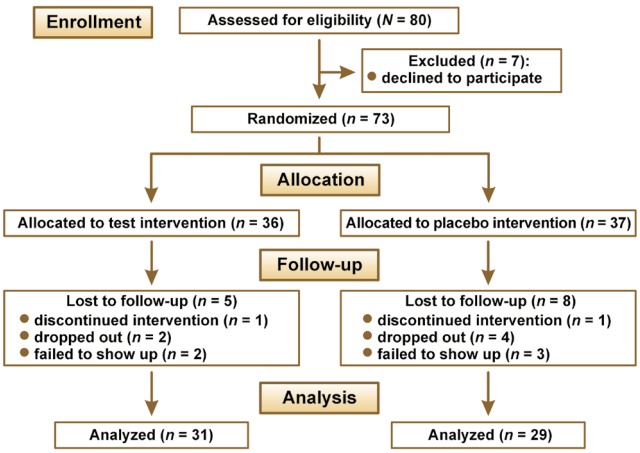

Fig. 1. Flowchart of the study

Intervention

Each child received a jar containing 2 tablets to take daily for 3 months. The active ingredients were the powdered cranberry extract (*Vaccinium macrocarpon*), standardized to 40% proanthocyanidins, and inactivated *L. salivarius* CECT 5713 at 10 mg, equivalent to 1×10^9 colony-forming units (CFU). The exact tablet composition is detailed in the supplementary material (available on request from the corresponding author). Placebo tablets were identical in size, color, texture, and sweetness, but contained no active ingredients. The tablets were packaged, coded and supplied by NutroPharma (Lesznowola, Poland). The parents were instructed to administer the tablets in the evening after tooth brushing, and the children were encouraged to let them dissolve slowly in the mouth to maximize tooth contact. Throughout the intervention, the parents were advised against giving their children dairy products containing probiotics, but they could provide traditionally fermented products, such as natural yogurt, kefir or buttermilk. In addition, the parents were asked not to provide any foods or sweets containing xylitol, and to refrain from fluoride exposure other than fluoridated toothpaste (~1,000 ppm, in the mornings and evenings). No specific remineralizing agents were used. During the study, the children received restorative dental treatment for cavitated lesions based on individual clinical needs and ethical considerations. Such treatment impacts the dmft index, but not the International Caries Detection and Assessment System (ICDAS) II score, which exclusively assesses the presence and extent of carious lesions.

Saliva sampling and microbial examinations

We quantified the salivary levels of *S. mutans* and *Lactobacillus* spp. through conventional cultivation. Paraffin-stimulated whole saliva was collected over 5 min and the samples were serially diluted in phosphate-buffered saline (PBS). Aliquots of 0.1 mL from serial dilutions were inoculated in duplicate onto selective agar plates from BioMaxima (Lublin, Poland): TYCSB (tryptone yeast extract cystine with sucrose and bacitracin) agar for *S. mutans* and MRS (de Man– Rogosa–Sharpe) agar for *Lactobacillus* spp. The agar plates were inoculated using a sensor turntable (Sensorturn pro; WLD-TEC, Arenshausen, Germany) and incubated in an anaerobic environment at 37°C for 5 days. The mean number of CFU for each suspension and dilution was counted, and we expressed the total bacterial load per 1 mL of saliva.

Collection of background data and clinical examinations

At baseline, we collected socioeconomic and medical background data from the parents, as well as information on the child's oral health behavior and dietary preferences, using predetermined questions. Clinical examinations were conducted in a dental office under optimal illumination by means of a WHO-621 probe. The children refrained from eating and drinking for 2 h before the examination, and did not brush their teeth within 12 h before the appointment. All dental surfaces in subsequent quadrants were examined, and dental caries was scored after cleaning and air-drying according to the ICDAS II criteria at baseline and after 9 months by 3 experienced examiners (A.T.S., P.P.Z., J.G.).

Caries prevalence at baseline and the 9-month incidence were calculated as the mean values of initial (ICDAS 1+2), moderate (ICDAS 3+4) and extensive (ICDAS 5+6) lesions. For the dmft index, ICDAS II scores of 3–6 were considered as "decayed". To express the baseline caries prevalence, the dmft index captured and described the total burden of the disease (decayed, missed and filled teeth) to indicate the enrollment of a truly caries-active child population. The incidence of new lesions during the study was scored using the ICDAS II index to capture the number of new early (non-cavitated), moderate and extensive lesions, allowing for a detailed description of lesion severity.

Inter- and intra-examiner reliability was assessed by examining 6 randomly selected 5-year-old children with ECC before the study. The results showed very good intra-examiner agreement for the ICDAS II scores (κ = 0.89, 0.79 and 0.91). The interrater reliability was also excellent for the 3 examiners, with kappa values ranging from 0.83 to 0.94.

The oral hygiene levels were determined using the simplified oral hygiene index (OHI-S). The buccal and labial surfaces of 6 teeth were scored after the application of a disclosing solution containing 3% erythrosine. The plaque scores for each individual were summed and divided by the number of the registered surfaces.

Outcome measures

The primary outcome was caries incidence over a 9-month period. Secondary endpoints were the level of oral hygiene and the counts of *S. mutans* and *Lactobacillus* spp. in stimulated saliva. For the primary endpoint, the null hypothesis was that caries incidence in the active group would not differ from that in the placebo group.

Assessment of compliance and side effects

The parents were asked to return the tablet packs at the 3-month follow-up to monitor intake. Compliance was categorized as "good" if ≤3 tablets were missed within a week, and "doubtful" if missed more often. The parents were also encouraged to report any perceived adverse or side effects and, in such cases, discontinue tablet use.

Statistical analysis

All data was stored at the Department of Pediatric Dentistry, Medical University of Warsaw. The data was analyzed using the Statistica 13 package (TIBCO Software Inc., Palo Alto, USA). Continuous data was compared between the groups using parametric (*t* test) and non-parametric (Mann–Whitney *U* test) tests if variables did not follow a normal distribution. Categorical data and proportions were compared using the χ^2 test. Inter- and intra-examiner agreement was assessed with Cohen's kappa coefficient, and *p*-values less than 0.05 were considered statistically significant.

Results

Sixty children completed the trial, giving an attrition rate of 19%. The reasons for dropout are shown in Fig. 1. There were no significant differences between the groups at baseline regarding the socioeconomic status, oral health behavior or diet, except for a higher proportion of fathers with primary or vocational education in the placebo group (Table 1). The baseline caries prevalence, expressed as dmft, was 71% in the test group and 72% in the placebo group, with the mean values of 7.0 ±4.5 in both groups. The baseline ICDAS II values and the 9-month caries incidence for initial, moderate and severe lesions are presented in Table 2.

A significantly lower incidence of initial lesions (ICDAS 1+2) was observed in the test group as compared to the placebo group after 9 months ($p < 0.05$). Although the incidence of moderate lesions (ICDAS 3+4) was also lower in the test group, the difference did not reach statistical significance. The intervention had no significant effect on the level of oral hygiene (OHI-S); the baseline value of 0.8 ±0.4 remained unchanged throughout the trial in both groups. Similarly, there were no statistically significant changes in the salivary counts of lactobacilli or *S. mutans* (Table 3). Compliance with the study protocol

Table 1. Baseline characteristics of the study groups

Variable			Test group ($n = 31$)	Placebo group ($n = 29$)	*p*-value
Parents	self-assessed socioeconomic status	high	3%	14%	NS
		average	94%	76%	NS
		low	3%	10%	NS
	mother's educational level	high	68%	69%	NS
		secondary	19%	24%	NS
		primary/vocational	13%	7%	NS
	father's educational level	high	55%	48%	NS
		secondary	35%	17%	NS
		primary/vocational	10%	35%	<0.05*
	mother's age [years]	>40	7%	3%	NS
		31–40	90%	87%	NS
		<31	3%	10%	NS
Children	age [years] ($M \pm SD$)		4.6 ±1.0	4.5 ±0.9	NS
	tooth brushing at least twice per day		77%	79%	NS
	tooth brushing by an adult		10%	7%	NS
	tooth brushing with an adult's supervision		71%	79%	NS
	intake of sweet foodstuff (more than once daily)		58%	48%	NS
	daily number of snacks ($M \pm SD$)		2.6 ±0.9	2.2 ±1.2	NS

M – mean; *SD* – standard deviation; NS – non-significant; * statistically significant (*t* test and χ^2 test).

Table 2. Mean caries prevalence at baseline and the incidence of new lesions during the study

Variable	Time point	Test group (n = 31)	Placebo group (n = 29)	p-value
ICDAS 1+2 (initial lesions)	baseline	3.4 ±2.6	4.3 ±3.6	NS
	9 months	0.3 ±0.6	1.0 ±0.7	<0.05*
ICDAS 3+4 (moderate lesions)	baseline	0.3 ±0.7	0.7 ±0.8	NS
	9 months	0.4 ±0.6	0.7 ±0.7	NS
ICDAS 5+6 (extensive lesions)	baseline	0.2 ±0.8	0.4 ±0.9	NS
	9 months	0.1 ±0.3	0.1 ±0.2	NS

Data presented as *M* ±*SD*.
ICDAS – International Caries Detection and Assessment System; NS – non-significant; * statistically significant (Mann–Whitney *U* test).

Table 3. *Lactobacillus* spp. and *Streptococcus mutans* counts in stimulated saliva [CFU/mL] at baseline and follow-up

Variable	Time point	Test group (n = 31)	Placebo group (n = 29)	p-value
Lactobacillus spp.	baseline	2.4×10^7 ±4.0×10^7	2.9×10^7 ±4.2×10^7	NS
	3 months	4.8×10^7 ±5.8×10^7	4.4×10^7 ±6.6×10^7	NS
	9 months	2.6×10^7 ±4.0×10^7	3.1×10^7 ±4.2×10^7	NS
S. mutans	baseline	7.1×10^6 ±9.8×10^6	4.8×10^6 ±5.6×10^6	NS
	3 months	5.7×10^6 ±5.6×10^6	9.1×10^6 ±1.0×10^6	NS
	9 months	7.1×10^6 ±9.5×10^6	5.0×10^6 ±5.5×10^6	NS

Data presented as *M* ±*SD*.
NS – non-significant (Mann–Whitney *U* test).

was good among all children who completed the trial, with a maximum of 2 missed tablets per week. No side effects or adverse events were reported by the parents or children.

Discussion

This study evaluated the combined effect of a paraprobiotic strain and the cranberry extract on the development of caries and salivary bacterial counts in preschool children over a 9-month period. To the best of our knowledge, this combination has not been investigated so far in a placebo-controlled trial. The use of organic products and natural polymers to prevent disease and maintain health aligns with the concept of green dentistry.[16,17] The rationale behind this combination of natural pre- and probiotic agents was that its synergistic effects could potentially be more powerful than the action of the agents used individually. Cranberry polyphenols (proanthocyanidins) may inhibit the production of organic acids and the formation of dysbiotic biofilm by hindering bacterial adhesion to tooth surfaces. They may also beneficially modulate the microbial ecology of dental plaque in high-caries-risk patients.[13,18–21] In addition, cranberry polyphenols may affect the production and activity of proteolytic enzymes that contribute to the destruction of the extracellular matrix in dental biofilm.[22] We selected a specific high-molecular-weight cranberry extract, previously shown to significantly reduce salivary *S. mutans* counts in children.[23] The ability of probiotic supplements to lower the counts of caries-associated microorganisms in saliva and dental biofilm through the production of bacteriocins, and competition for adhesion and nutrients is well established.[24] In this context, paraprobiotic and probiotic strains derived from *Lactobacillus* spp. appear particularly promising for clinical applications in maintaining oral health.[25,26] The strain used in this trial, *L. salivarius* CECT 5317, is a non-active strain isolated from human milk.[10] The main finding of this study was that the combination of pre- and probiotics significantly reduced the incidence of initial carious lesions after 9 months of a daily intake. Although there were fewer new moderate lesions in the test group, this difference did not reach statistical significance. Consequently, we rejected the null hypothesis.

For all stages of carious lesions, the reduction in new carious lesions was 44%, a magnitude comparable to previous trials using live probiotic bacteria in preschool children.[6] This clinical effect is particularly significant given that the participating children were selected for being caries-active. For comparison, Rodríguez et al. reported a 50% reduction in the incidence of cavitated lesions among caries-active preschool children enrolled in a 1-year program of a daily milk intake supplemented with *Lactobacillus rhamnosus* SP1.[27] To our knowledge, only one previous trial has evaluated the effect of thermally inactivated probiotics on the development of caries in preschool children.[11] In that study, despite a short intervention period of only 14 days, a reduced incidence of ECC was observed after 12 months as compared to "treatment as usual".[11] Collectively, the findings from our study and the aforementioned research suggest that inactivated *L. salivarius* strains may have anti-caries properties similar to those of live probiotic bacteria. This information is important for the development, production, storage, and shelf life of future consumer products.

With regard to saliva, numerous studies with probiotic supplements have reported an immediate but short-term decrease in *S. mutans*, whereas *Lactobacillus* counts are often slightly increased.[28] Surprisingly, in our study, we did not observe any effect on salivary bacterial counts, which contrasts with earlier reports evaluating live or inactivated strains of *L. salivarius*.[10,29–31] However, previous studies relied on biofilm models,[30,31] involved short-term protocols with healthy volunteers[10] or used simple chair-side tests.[29] We found no effect of the intervention on the level of oral hygiene, assessed using OHI-S, which is consistent with many previous reports on probiotic supplements.[32] Cranberries, however, contain phenolic compounds that may

disrupt biofilm formation, so an effect on biofilm accumulation would not have been surprising. It should, however, be noted that the mean OHI-S score was relatively low (<1.0) among the participating children, which did not necessarily reflect their high cariogenic challenge. This observation underscores the idea that the presence of "virulence hotspots", rather than the amount of biofilm, is decisive for local caries activity in young children.[33]

Strengths and limitations

The strengths of this study include its strict randomized, placebo-controlled, double-blind longitudinal cohort design, and high compliance. However, there were limitations, including a relatively high dropout rate, although evenly distributed between the groups. The post hoc power analysis indicated strong statistical power (98.6%). Notably, we presented our data per protocol, and the significant findings persisted in the intention-to-treat analysis. However, we could not investigate the separate contribution of each active ingredient (*V. macrocarpon* and *L. salivarius*) with the current study design, as it would have required 4 parallel study arms and a larger sample size. We consider the clinical scoring reliable given the very good inter- and intra-examiner agreement. However, the external validity of our findings may be limited, since the study group represented a selected high-caries population. Therefore, any generalization of these findings to populations with a lower ECC burden should be approached cautiously. Nevertheless, the outcomes of this trial encourage and justify further research into the anti-caries role of this synbiotic mix containing paraprobiotics and natural cranberry polyphenols.

Conclusions

A daily intake of tablets containing a combination of a paraprobiotic strain and the cranberry extract significantly reduced the 9-month incidence of initial non-cavitated carious lesions in caries-active preschool children in comparison with placebo.

Trial registration

The project was registered at ClinicalTrials.gov (NCT 03919838).

Ethics approval and consent to participate

The study was conducted ethically in accordance with the World Medical Association (WMA) Declaration of Helsinki, and approved by the Bioethics Committee of the Medical University of Warsaw, Poland (No. KB/232/2016). Written informed consent for participation was collected from all participants' parents/legal guardians prior to involvement.

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.

ORCID iDs

Dorota Olczak-Kowalczyk https://orcid.org/0000-0002-1567-3844
Anna Turska-Szybka https://orcid.org/0000-0003-0248-6625
Svante Twetman https://orcid.org/0000-0002-0199-9210
Dariusz Gozdowski https://orcid.org/0000-0002-7365-7607
Paula Piekoszewska-Ziętek https://orcid.org/0000-0003-0968-8529
Joanna Góra https://orcid.org/0009-0005-7293-6080
Marta Wróblewska https://orcid.org/0000-0002-9126-7442

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Original paper

Effects of chlorhexidine gluconate and povidone-iodine mouthwash on cycle threshold values in patients infected with SARS-CoV-2

Lilies Dwi Sulistyani[1,C–F], Teuku Zulfahmi Rizki[1,A–D], Budi Haryanto[2,C,E], Vera Julia[1,E], Arfan Badeges[3,E], Dwi Ariawan[1,E], Mohammad Adhitya Latief[1,E], Yudy Ardilla Utomo[1,E]

[1] Department of Oral and Maxillofacial Surgery, University of Indonesia, Jakarta, Indonesia
[2] Department of Clinical Microbiology, Persahabatan Central General Hospital, Jakarta, Indonesia
[3] Department of Oral and Maxillofacial Surgery, Persahabatan Central General Hospital, Jakarta, Indonesia

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):217–223

Address for correspondence
Lilies Dwi Sulistyani
E-mail: liliesdwi_s@yahoo.co.id

Funding sources
The study was funded by the University of Indonesia PUTI (Internationally Indexed Publication) research grant No. NKB-240/UN2.RST/HKP.05.00/2022).

Conflict of interest
None declared

Acknowledgements
The authors would like to thank the University of Indonesia for the financial support.

Received on June 5, 2024
Reviewed on July 23, 2024
Accepted on August 20, 2024

Published online on April 30, 2025

Cite as
Sulistyani LD, Rizki TZ, Haryanto B, et al. Effects of chlorhexidine gluconate and povidone-iodine mouthwash on cycle threshold values in patients infected with SARS-CoV-2. *Dent Med Probl.* 2025;62(2):217–223. doi:10.17219/dmp/192493

DOI
10.17219/dmp/192493

Abstract

Background. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants exhibit different phenotypes and clinical manifestations in comparison to non-mutated viruses. Spike gene target failure (SGTF) is a characteristic feature of the gene in a novel variant that is recognized as highly transmissible. Several studies have demonstrated the virucidal effects of mouthwashes on SARS-CoV-2. Moreover, mouthwashes have proven beneficial for patients undergoing oral and maxillofacial surgery.

Objectives. The present study aimed to analyze the effects of 2 different types of mouthwash (0.2% chlorhexidine gluconate and 1% povidone-iodine) on the cycle threshold (CT) values in coronavirus disease 2019 (COVID-19) patients with and without SGTF.

Material and methods. This single-blind, non-randomized controlled clinical trial comprised 45 patients who were divided into 3 groups based on the intervention method: 0.2% chlorhexidine gluconate mouthwash; 1% povidone-iodine mouthwash; and mineral water (control group). The patients were instructed to gargle with the assigned solution 3 times a day for 5 days. Reverse transcription polymerase chain reaction (RT-PCR) tests were conducted at the time of initial diagnosis and on days 3 and 5. A normality test (Shapiro–Wilk test) was performed. Consequently, the non-parametric Friedman test was used.

Results. The analysis revealed that the subjects who utilized mouthwashes exhibited higher CT values in comparison to the control group. Furthermore, 73% of patients who used 0.2% chlorhexidine gluconate presented with increased CT values, as indicated by a negative RT-PCR test on the 3rd day.

Conclusions. Gargling with 0.2% chlorhexidine gluconate or 1% povidone-iodine for 30 s for at least 3 days has been demonstrated to increase CT values in both SGTF and non-SGTF COVID-19 patients. Hence, using the mouthwash may be considered for preoperative use in patients undergoing oral and maxillofacial surgery.

Keywords: mouthwash, COVID-19, chlorhexidine, povidone-iodine, CT value

Highlights

- The findings of the present study suggest that gargling with a 0.2% chlorhexidine gluconate or a 1% povidone-iodine mouthwash significantly increases RT-PCR cycle threshold (CT) values, indicating a measurable reduction in SARS-CoV-2 viral load.
- Notably, 73% of COVID-19 patients using a chlorhexidine mouthwash tested negative by day 3, demonstrating faster viral suppression as compared to other treatment groups.
- The mouthwash intervention proved effective in both SGTF (variant) and non-SGTF (non-variant) COVID-19 cases, underscoring its broad-spectrum antiviral potential.
- These results highlight the potential benefits of antiviral oral rinses in reducing the risk of viral transmission, particularly in oral and maxillofacial surgery settings.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly infectious virus that primarily affects the respiratory tract. The virus is transmitted via breathing, coughing or sneezing; additionally, it can be disseminated through direct contact with contaminated surfaces and then touching the nose, mouth and eyes.[1–3] Reverse transcription polymerase chain reaction (RT-PCR) is a diagnostic test used for detecting the presence of SARS-CoV-2.[4,5]

Different variants of SARS-CoV-2 exhibit different characteristics. The Omicron variant, a novel mutated form of SARS-CoV-2 known as B.1.1.529, has been designated as a variant of concern by the World Health Organization (WHO).[6,7] Omicron demonstrates high transmissibility, spreading more rapidly than other variants.

Several RT-PCR protocols have been used to describe the characteristics of specific variants of SARS-CoV-2. The spike glycoprotein (*S*) gene is used to detect the SARS-CoV-2 variant.[8,9] Spike gene target failure (SGTF), which refers to a failure to detect this gene, has been observed in patients with the Omicron variant. In contrast, non-SGTF coronavirus disease 2019 (COVID-19) patients often present with other types of SARS-CoV-2 variants.[6] The classification of the SARS-CoV-2 variant as SGTF or non-SGTF provides greater specificity and can serve as an initial screening method for the identification of the SARS-CoV-2 variant that has this mutation.

Mouthwash has been used to prevent the transmission of SARS-CoV-2 prior to dental treatment, including oral and maxillofacial surgery. According to Huang and Huang, chlorhexidine gluconate effectively (86.0%) reduced SARS-CoV-2 in the oropharynx.[10] The Centers for Disease Control and Prevention (CDC) recommend using a povidone-iodine-based mouthwash before any treatment in the oral cavity.[11] In another study, 1% povidone-iodine suppressed the load of the SARS-CoV-2 in the oral cavity.[12]

However, direct clinical trials on SGTF and non-SGTF COVID-19 patients are limited. The aim of this study was to analyze the effects of 2 types of mouthwash (0.2% chlorhexidine gluconate and 1% povidone-iodine) on the cycle threshold (CT) values in RT-PCR tests in patients with all variants of SARS-CoV-2.

Material and methods

Ethical clearance

The study protocol was reviewed and approved by the Health Ethics Committee of Persahabatan Central General Hospital, Jakarta, Indonesia (protocol No. 73/KEPK-RSUPP/08/2022).

Study design and intervention

The study was performed at Persahabatan Central General Hospital in August 2022. This single-blind, non-randomized controlled clinical trial comprised 45 patients who were divided into 3 intervention groups: a 0.2% chlorhexidine gluconate mouthwash group (n = 15); a 1% povidone-iodine mouthwash group (n = 15); and a mineral water control group (n = 15). The mouthwash was repackaged in 125-mL bottles. Each subject received 2 bottles of mouthwash (250 mL in total).

Patients who met the inclusion criteria and were being treated at the oral and maxillofacial surgery clinic underwent RT-PCR examination. The collection of sample material for RT-PCR was carried out by trained personnel in the microbiology laboratory at Persahabatan Central General Hospital. No specific time for sample collection was stipulated. The patients were instructed to gargle with 15 mL of a mouthwash (30 s in the oral cavity and 30 s in the back of the throat) 3 times a day for 5 days. Subsequent to gargling, the subjects were asked to rinse their mouth with 15 mL of water. Observations were carried out via video call for each gargle. Reverse transcription polymerase chain reaction examinations were performed to obtain CT values at baseline and on days 3 and 5. All sample materials for RT-PCR were taken from the oropharyn-

geal swabs using a disposable virus sampling tube (Baicare Biotechnology Co., Ltd., Beijing, China). The specimens were then vortexed with an LMS® UZUSIO VTX-3000L vortex mixer (LMS Co., Ltd., Tokyo, Japan) for 20 s and left to stand for 15 min. The IVD Reagent MAD-003941M (Vitro Master Diagnostica®, Madrid, Spain) was mixed with 200 µL of the specimen. The cartridge was loaded into the MagNA Pure 96 instrument (Roche, Basel, Switzerland) for sample extraction. The reaction mixture of the mBioCoV-19 RT-PCR Kit (Bio Farma, Bandung, Indonesia) was used for the detection of open reading frame 1b and RNA-dependent RNA polymerase genes. In brief, 15 µL of the reaction mix were added to each well and subsequently mixed with 5 µL of the extracted specimen. Cycle threshold values were obtained automatically upon the detection of SARS-CoV-2 genetic material with the use of an Exicycler™ 96 (v. 4) (RRID:SCR_022144) Real-Time Quantitative Thermal Block (Bioneer Corporation, Daejeon, South Korea). The use of this reagent in all samples enabled the measurement of CT values and the identification of *S* gene targets. Samples without the *S* gene were categorized as SGTF, while those containing the *S* gene were classified as non-SGTF.

Sample population

Prior to enrollment in the study, the patients were subjected to a screening process that evaluated their eligibility based on a set of predetermined criteria. The inclusion criteria were as follows: SARS-CoV-2-positive patients, as confirmed by RT-PCR results within the previous 3 days; an RT-PCR CT value of ≤30; outpatients with mild or no symptoms; and an age range of 20–50 years. Patients with comorbidities, those with a history of allergy to povidone-iodine mouthwash and chlorhexidine gluconate, pregnant females, and those who were not willing to participate were excluded from the study.

Table 1. Characteristics of the study sample ($N = 45$)

Variable		Patients *n* (%)
Sex	male	22 (48.9)
	female	23 (51.1)
Age [years]	20–30	16 (35.6)
	31–40	18 (40.0)
	41–50	11 (24.4)
SARS-CoV-2 variant	SGTF	34 (75.6)
	non-SGTF	11 (24.4)

SGTF – spike gene target failure; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2.

Sample size

The sample size was determined using the G*Power v. 3.1.9.7 software (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower), and the estimated results were 15 patients in each group.

Statistical analysis

The Shapiro–Wilk test was used to check for the normality of data. Then, the non-parametric Friedman test and the post hoc Wilcoxon test were used to evaluate the CT values. The data was analyzed using the IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA). A value of $p < 0.05$ was considered statistically significant.

Results

Characteristics of patients

As shown in Table 1, 51.1% ($n = 23$) of the patients were female and the remaining 48.9% ($n = 22$) were male. The age of the patients ranged from 21 to 48 years, and 40.0% of the patients belonged to the 31–40 age group. Furthermore, 34 patients (75.6%) were identified with SGTF, while the remaining 11 (24.4%) belonged to the non-SGTF group.

CT values

Statistically significant differences in CT values ($p < 0.05$) were observed in all 3 groups following gargling at baseline and on days 3 and 5 (Table 2). A statistically

Table 2. Cycle threshold (CT) values in the study sample based on the mouthwash group

Group	Patients, *n*	CT value *M* ±*SD*			*p*-value
		baseline	3rd day	5th day	
0.2% chlorhexidine gluconate	15	23.51 ±4.03	37.66 ±4.33	39.61 ±1.50	<0.001*
1% povidone-iodine	15	24.34 ±4.19	33.32 ±7.47	37.43 ±4.74	<0.001*
Control	15	27.80 ±6.39	32.92 ±7.10	35.18 ±6.05	<0.001*
Total	45	25.22 ±5.22	34.63 ±6.67	37.41 ±4.78	<0.001*

* statistically significant ($p < 0.05$, Friedman test); *M* – mean; *SD* – standard deviation.

significant difference was observed based on the intervention time, indicating that CT values increased on a daily basis. The highest average increase in CT values (14.15) was observed from baseline to day 3 in the 0.2% chlorhexidine gluconate mouthwash group. A total of 73% of patients who used 0.2% chlorhexidine gluconate presented with increased CT values, as indicated by a negative RT-PCR test on the 3rd day. The 1% povidone-iodine mouthwash group exhibited an average increase of 4.11 from day 3 to day 5 (Fig. 1). Similarly, significant increases in CT values were observed in all 3 groups following gargling in SGTF and non-SGTF COVID-19 patients (Table 3). The CT values in the SGTF and non-SGTF groups exhibited a significant increase on a daily basis until day 5.

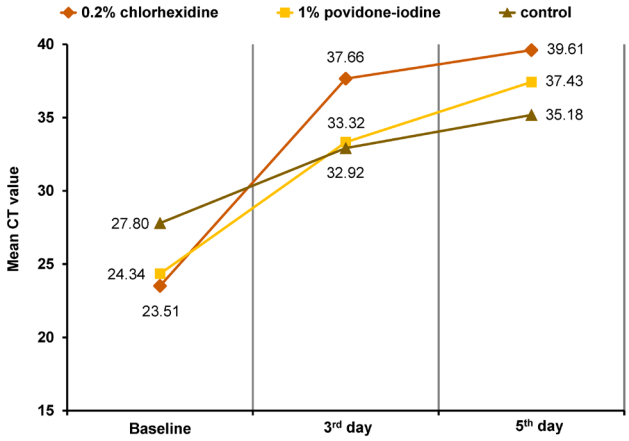

Fig. 1. Increase in the mean cycle threshold (CT) values of the patients among the 3 groups at 3 time points

Discussion

Severe acute respiratory syndrome coronavirus 2, which caused the global pandemic of COVID-19, has infected more than 627 million people worldwide and more than 6.4 million Indonesians until October 2022.[13] At the time of this study, there were 2,087 active cases of the virus in Indonesia. The majority of the patients in the current study were in the 31–40 age group, with an overall age range of 21–48 years. These findings are consistent with those reported by Megasari et al.[14] As reported by Karyono et al., 80% of patients infected with SARS-CoV-2 exhibited mild symptoms, and 18% of patients were asymptomatic. Consequently, the patients were unaware of their infection, which resulted in the transmission of the virus to health workers.[15]

Several studies have been conducted to establish the most effective prevention protocol against SARS-CoV-2. The present study aimed to evaluate the preventive effects of 0.2% chlorhexidine gluconate and 1% povidone-iodine mouthwashes on patients infected with SARS-CoV-2. Many viruses present in the oral cavity and upper respiratory tract can be transmitted through various means, including speech, sneezing or coughing. These pathogens can also be disseminated during medical procedures performed in the oral cavity. Shankar et al. stated that the upper respiratory tract plays the most important role in the transmission of SARS-CoV-2.[12] Likewise, Karyono et al. reported the presence of the virus in the oral cavity, particularly the saliva, and further noted that the viral load in saliva at the onset of the infection was higher than that in the oropharynx.[15] Gargling with mouthwash has been shown to reduce the number of viruses in the oral cavity and at the back of the throat. The decrease in the number of viruses can be estimated by the CT value in the RT-PCR test, which indicates the concentration of the genetic material of the virus in a specimen.[16,17] Chlorhexidine gluconate and povidone-iodine mouthwashes have antibacterial and antiviral properties; they are commonly used and readily available. In studies by Boyapati et al.[18] and Soundarajan and Rajasekar,[19] chlorhexidine was considered the gold standard antibacterial mouthwash when compared to other novel types of mouthwash, such as probiotic mouthwash[18] and amla seed-mediated graphene oxide-silver (GO-Ag) nanocomposite mouthwash.[19]

Table 3. Cycle threshold (CT) values in the spike gene target failure (SGTF) and non-SGTF patients

Group		Patients, n	CT value *M ±SD*			*p*-value
			baseline	3rd day	5th day	
SGTF	0.2% chlorhexidine gluconate	12	22.31 ±4.45	35.45 ±4.37	37.21 ±1.67	<0.001*
	1% povidone-iodine	12	22.24 ±4.32	32.35 ±7.56	36.83 ±4.23	<0.001*
	control	10	25.31 ±6.37	31.97 ±7.17	34.29 ±6.65	<0.001*
Non-SGTF	0.2% chlorhexidine gluconate	3	23.65 ±3.23	34.42 ±3.45	36.28 ±1.47	<0.001*
	1% povidone-iodine	3	23.53 ±4.36	31.65 ±6.14	35.68 ±3.41	<0.001*
	control	5	24.82 ±5.38	32.51 ±5.24	33.41 ±7.41	<0.001*

* statistically significant ($p < 0.05$, Friedman's test).

The standard molecular method for the diagnosis of COVID-19 is RT-PCR. The CT value describes the number of amplification cycles required for the target gene to exceed the threshold level during RT-PCR examination. Therefore, CT values are inversely proportional to viral load, thereby serving as an indirect method for calculating the number of copies of viral ribonucleic acid (RNA) present in a sample.[16]

As illustrated in Fig. 1, the study demonstrated an increase in CT values in patients who gargled with the 2 types of mouthwash.[20] This finding aligns with the results of previous studies, which demonstrated the effectiveness of chlorhexidine gluconate on SARS-CoV-2.[21] Yoon et al. reported a decrease in the SARS-CoV-2 viral load in saliva after gargling with 0.2% chlorhexidine gluconate.[22] Other studies have shown that 0.2% chlorhexidine gluconate can reduce the risk of SARS-CoV-2 transmission via aerosols.[23,24] In the present study, the highest increase in CT values was observed in the group that gargled with 0.2% chlorhexidine gluconate within the first 3 days (Table 4) compared to that in the 1% povidone-iodine group (14.15 vs. 8.98, respectively). There was a statistically significant difference in the first 3 days for all groups. The reason for the observed differences in the effectiveness of the 2 types of mouthwash remains unclear.

Table 2 demonstrates statistically significant differences in the mean CT values between the 2 types of mouthwash. The findings indicate that 1% povidone-iodine mouthwash can be used as an alternative to 0.2% chlorhexidine gluconate to effectively increase CT values. The American Dental Association (ADA) and the CDC have recommended using 1% povidone-iodine mouthwash before performing any procedures in the oral cavity, including those pertaining to oral and maxillofacial surgery. Furthermore, gargling with 1% povidone-iodine or 0.2% chlorhexidine gluconate has been shown to reduce the load of SARS-CoV-2 in the upper respiratory tract, thereby increasing the CT value. Gargling instigates a water cycle that mechanically washes away viruses and other infected cells adhered to the cilia in the epithelial mucosa of the oral cavity and throat.[25] In the study by Robinot et al., SARS-CoV-2 infection in ciliated epithelial cells resulted in a loss of ciliary motility.[26] The cilia in patients infected with SARS-CoV-2 were found damaged and shortened after gargling with 1% povidone-iodine and 0.2% chlorhexidine gluconate.

The control group in this study showed a statistically significant increase in CT values in RT-PCR, consistent with the findings of Satomura et al., who demonstrated that gargling with water at the oropharynx area 3 times a day effectively reduced the incidence of upper respiratory tract infections by 36%.[25] Rinsing the upper respiratory tract, which mechanically removes excess mucus, is beneficial for patients infected with SARS-CoV-2. Gargling generates a swirl of water that mechanically removes viruses and virus-infected cells from the oral cavity and back of the throat.[21,22] This action has been shown to decrease viral load and increase CT values in RT-PCR.[26]

The subjects of this study were divided into 2 groups based on the detection of the *S* gene from the RT-PCR results. A mutation in the *S* gene due to a deletion of the H69-V70 amino acids results in the failure of detection of the *S* gene, or SGTF. This H69-V70 amino acid deletion has been identified in several variants of SARS-CoV-2, including the Omicron variant.[6,8] Significant differences in the increases in CT values were observed after gargling with 0.2% chlorhexidine gluconate and 1% povidone-iodine in both the SGTF and non-SGTF groups (Table 4). These results indicate that these 2 types of mouthwash are effective against all variants of SARS-CoV-2. Hence, gargling can be implemented as a supportive or complementary therapy within the COVID-19 treatment protocol. Beyond its role in mitigating the transmission of SARS-CoV-2, gargling with antimicrobial mouthwash prior to dental and oral surgery procedures has the potential to enhance oral hygiene and reduce gingival and periodontal inflammation. This, in turn, may lead to a reduction in the incidence of complications arising from COVID-19.[27] In the context of future studies, other types of antimicrobial mouthwash, such as the probiotic mouthwash, could be evaluated for its efficacy in increasing the CT value of SARS-CoV-2, as reported in the study by Boyapati et al.[18] The efficacy of probiotic mouthwash in treating chronic gingivitis has been demonstrated to be comparable.[18]

Table 4. Comparison of cycle threshold (CT) values at different time points

Group	Patients, *n*	Difference in mean CT values					
		3rd day–baseline		5th day–3rd day		5th day–baseline	
		difference	*p*-value	difference	*p*-value	difference	*p*-value
0.2% chlorhexidine gluconate	15	14.15	<0.001*	1.95	0.068	16.10	<0.001*
1% povidone-iodine	15	8.98	<0.001*	4.11	0.018*	13.09	<0.001*
Control	15	5.12	<0.001*	2.26	0.008*	7.38	<0.001*

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

Conclusions

Gargling with 0.2% chlorhexidine gluconate or 1% povidone-iodine for 30 s for at least 3 days was shown to reduce the viral load of SARS-CoV-2. An increase in CT values was observed in patients with and without SGTF, indicating that mouthwash is effective against all SARS-CoV-2 variants. Gargling with 0.2% chlorhexidine gluconate or 1% povidone-iodine could be considered as an initial protocol prior to oral and maxillofacial surgical procedures in patients with COVID-19.

Trial registration

This study was registered in the International Standard Randomised Controlled Trial Number (ISRCTN) registry under No. ISRCTN13090248.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Health Ethics Committee of Persahabatan Central General Hospital, Jakarta, Indonesia (protocol No. 73/KEPK-RSUPP/08/2022).

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.

ORCID iDs

Lilies Dwi Sulistyani https://orcid.org/0000-0001-5542-6787
Teuku Zulfahmi Rizki https://orcid.org/0000-0001-9839-6733
Budi Haryanto https://orcid.org/0009-0001-5249-9945
Vera Julia https://orcid.org/0000-0001-6182-5688
Arfan Badeges https://orcid.org/0000-0003-0786-7750
Dwi Ariawan https://orcid.org/0000-0001-6407-4576
Mohammad Adhitya Latief https://orcid.org/0000-0002-8009-4718
Yudy Ardilla Utomo https://orcid.org/0000-0001-8590-2983

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Original paper

Circulating biomarkers of nitrosative stress, protein glycoxidation and inflammation in maxillofacial surgery patients treated with titanium implants

Bożena Antonowicz[1,A–D,F], Jan Borys[2,B], Anna Zalewska[3,A,C,F], Małgorzata Żendzian-Piotrowska[4,C], Kamila Łukaszuk[2,B], Łukasz Woźniak[1,B], Mariusz Szuta[5,C], Mateusz Maciejczyk[4,A–F]

[1] Department of Oral Surgery, Medical University of Bialystok, Poland
[2] Department of Maxillofacial and Plastic Surgery, Medical University of Bialystok, Poland
[3] Department of Conservative Dentistry, Medical University of Bialystok, Poland
[4] Department of Hygiene, Epidemiology and Ergonomics, Medical University of Bialystok, Poland
[5] Department of Oral Surgery, Jagiellonian University Medical College, Krakow, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):225–236

Address for correspondence
Mateusz Maciejczyk
E-mail: mat.maciejczyk@gmail.com

Funding sources
This work was supported by the Medical University of Bialystok, Poland (grant No. B.SUB.23.250; B.SUB.23.309).

Conflict of interest
None declared

Acknowledgements
None declared

Received on April 5, 2024
Reviewed on May 1, 2024
Accepted on May 18, 2024

Published online on January 31, 2025

Cite as
Antonowicz B, Borys J, Zalewska A, et al. Circulating biomarkers of nitrosative stress, protein glycoxidation and inflammation in maxillofacial surgery patients treated with titanium implants. *Dent Med Probl.* 2025;62(2):225–236. doi:10.17219/dmp/188863

DOI
10.17219/dmp/188863

Abstract

Background. Titanium (Ti) biomaterials are widely used in the surgical management of maxillofacial trauma, in oncology and orthognathic surgery. Although Ti is considered highly biocompatible, adverse reactions at the implant site have been reported in numerous clinical studies. However, the influence of Ti mandibular implants on glutathione metabolism, nitrosative stress and systemic inflammation has not been investigated to date.

Objectives. The study aimed to evaluate the acute (short-term) effects of Ti mandibular implants on the circulating biomarkers of the antioxidant defense system, on oxidative and nitrosative stress, as well as the inflammatory response of the blood plasma/erythrocytes, in maxillofacial surgery patients compared to the control group.

Material and methods. The experimental group consisted of 40 patients with bilateral mandibular fractures, who received osteosynthesis treatment with the use of Ti-6Al-4V alloy miniplates and screws. The control group comprised 40 age- and gender-matched patients who were qualified for the surgical treatment of craniofacial defects through bimaxillary osteotomy.

Results. An increase in the activity of pro-oxidant enzymes (↑ nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), ↑ xanthine oxidase (XO)), impaired glutathione metabolism (↓ total glutathione, ↑ oxidized glutathione (GSSG), ↓ reduced glutathione (GSH), ↓ redox status), higher levels of oxidative stress (↓ total thiols, ↑ malondialdehyde (MDA), ↑ lipid hydroperoxides (LOOHs)), ↓ total antioxidant status (TAS)), carbonyl stress (↑ dityrosine, ↑ N-formylkynurenine) and nitrosative stress (↑ nitric oxide (NO), ↑ S-nitrosothiols, ↑ peroxynitrite, ↑ nitrotyrosine), as well as an intensified systemic inflammatory response (↑ interleukin (IL)-1β, ↑ IL-6), were observed in maxillofacial surgery patients.

Conclusions. Despite the fact that the study examined only the circulating biomarkers of redox balance and inflammation, the results suggest that a systemic inflammatory response can be triggered by local immune reactions. Systemic inflammation and oxidative stress may stem from an early adaptive immune response to foreign objects in the body. Although further research is required, the removal of the existing Ti mandibular implants should be considered.

Keywords: inflammation, blood, nitrosative stress, titanium implants, circulating biomarkers

Highlights

- Maxillofacial surgery patients treated with Ti-6Al-4V show increased levels of the circulating biomarkers of oxidative stress, nitrosative stress and inflammation.
- A systemic inflammatory response can be triggered by local immune responses.
- The removal of Ti-6Al-4V implants in the craniofacial region should be considered.

Introduction

Titanium (Ti) and its alloys are the most widely used surgical biomaterials for the treatment and replacement of tissues and organs. Titanium implants are known for their high biofunctionality, biocompatibility, excellent biomechanical properties (durability, hardness and wear resistance), and the absence of a thrombotic response.[1,2] However, adverse reactions to Ti implants have been reported in numerous clinical studies.[3–5] The Ti-based prosthetic replacements of long bone joints have been found to induce inflammation, allergic responses and toxicity.[6,7] Additionally, grayish discoloration of the peri-implant tissues has been reported.[8,9] Metallic debris can be phagocytized by the circulating immune cells, which are stimulated to release pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6 and IL-8, and free radicals.[10,11] Side effects have also been observed in patients with dental implants.[12–14] Recent research indicates that there are differences in the response to Ti implants between the peri-implant soft tissue and the periodontal tissue.[15,16] Reactive oxygen species (ROS) are formed on the implant surface as a direct product of corrosion at the cathode, and from the interaction between the titanium dioxide (TiO_2) layer and hydrogen peroxide (H_2O_2) generated by the activated macrophages.[17–19] In turn, the chronic sterile inflammation caused by Ti implants in the surrounding tissues can lead to bone loss and prosthetic loosening.[20–22]

Titanium biomaterials are also commonly used in the surgical management of maxillofacial trauma, in oncology and orthognathic surgery.[23] Yet, recent research has confirmed the presence of Ti in the tissues surrounding mandibular implants.[24,25] Our previous studies revealed dysfunctions in enzymatic and non-enzymatic antioxidant defense systems, as well as protein and lipid damage caused by heightened oxidative and nitrosative stress, mitochondrial dysfunction, an intensified inflammatory response, and apoptosis, in patients who received osteosynthesis treatment with the use of Ti miniplates and screws.[26–28] However, the effects of Ti mandibular fixations on glutathione metabolism, nitrosative stress and systemic inflammation has not been studied to date. Titanium particles have been identified not only in the peri-implant tissues, but also in distal organs, such as lymph nodes, lungs, spleen, and liver.[4,29,30] The Ti ions have also been detected in the blood plasma of patients with Ti mandibular implants.[30,31] These observations clearly indicate the need for further research to expand our understanding of maxillomandibular implant degradation and its impact on systemic homeostasis, especially in the context of the ongoing debate on Ti implant removal.[32] Local redox imbalance, oxidative and nitrosative stress, and the inflammatory response may disrupt regenerative processes throughout the body.

Therefore, the aim of the present study was to evaluate the acute (short-term) effects of Ti mandibular fixations on the circulating biomarkers of the antioxidant defense system, on oxidative and nitrosative stress, and the inflammatory response of the blood plasma/erythrocytes in maxillofacial surgery patients compared to the control group. We assessed the activity of the main pro-oxidant enzymes (nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) and xanthine oxidase (XO)), the ROS scavenging capacity in the total antioxidant status (TAS) assay and the concentration of the main intracellular antioxidant, glutathione. In addition, we measured the concentrations of the most commonly assessed lipid peroxidation products (malondialdehyde (MDA) and lipid hydroperoxides (LOOHs)), and pro-inflammatory and anti-inflammatory cytokines (IL-1β, IL-6, IL-8, and IL-10).

Material and methods

Patients

The study involved 80 patients treated at the Department of Maxillofacial and Plastic Surgery of the Medical University in Bialystok, Poland. The study was approved by the Bioethics Committee of the Medical University in Bialystok (approval No. R-I-002/2/3/2-16). Patients in both the experimental and control groups were informed about the purpose of the study, the type and method of surgical treatment, and potential complications. All participants provided voluntary written informed consent to participate in the experiment.

The experimental group consisted of 40 patients (25 men and 15 women), aged 22–34 years, with bilateral mandibular fractures, who received osteosynthesis treatment with the use of Ti-6Al-4V alloy miniplates and screws (ChM Lewickie Sp. z o.o., Juchnowiec Kościelny, Poland). Bilateral mandibular corpus fractures were treated by fixation with 2 five-hole Ti miniplates and 4 screws per plate, with 1 patient receiving 4 miniplates and

16 screws. At the time of sustaining mandibular trauma, none of these patients had additional bone fractures or other organ injuries.

The control group comprised 40 patients (25 men and 15 women), aged 22–34 years, who were qualified for the surgical treatment of craniofacial defects through bimaxillary osteotomy.

The following exclusion criteria were applied: other bone fractures; a brain injury or other organ injuries; the presence of any Ti implants (dental, orthodontic, prosthetic, joint prostheses); chronic systemic or localized diseases; oral diseases, such as periodontitis; the inflammatory response triggered by periodontal disease (stomatitis, tonsillitis); the use of medications, supplements, psychoactive substances, or narcotics within 3 months before the commencement of the study; alcohol consumption; smoking or chewing tobacco; an unhealthy body mass index (BMI); abnormal results of biochemical analyses – blood morphology test, blood clotting parameters (prothrombin time (PT), activated partial thromboplastin time (APTT) and the international normalized ratio (INR)), the levels of electrolytes (sodium (Na) and potassium (K)), blood glucose, creatinine, urea, and liver enzymes (aspartate transaminase (AST) and alanine transaminase (ALT)).

In the experimental group, Ti miniplates and screws were removed at 3–4 months after surgery. No complications were observed during the process of fracture healing in any of the patients in the period between surgery and implant removal. There were no signs of inflammation, reddening, swelling, abscesses, allergic reactions, or implant exposure at the peri-implant site. The surgical procedures were performed by the same experienced maxillofacial surgeon (J.B.) in both groups.

Blood samples

In the experimental group, blood samples were collected 3–4 months after mandibular osteosynthesis, specifically on the day of miniplate and screw removal. In the control group, blood samples were collected before the bimaxillary osteotomy procedures.

Blood samples from both the experimental and control groups were obtained following overnight fasting and stored at 4°C. Plasma samples were separated via centrifugation at 3,000 rpm for 15 min and were subsequently stored at –80°C until biochemical analyses. Erythrocytes were rinsed 3 times with cold 0.9% NaCl solution and hemolyzed by the addition of a 9-fold volume of cold phosphate-buffered saline (PBS) (50 mM, pH 7.4).[33]

Biochemical analyses

The blood samples were analyzed to determine the activity of pro-oxidant enzymes, glutathione metabolism/TAS, the concentrations of protein and lipid oxidation products, glycoxidation products, nitrosative stress biomarkers, pro-inflammatory and anti-inflammatory cytokines, and total protein concentration.

All reagents for redox assays were supplied by either Sigma-Aldrich Chemie (Taufkirchen, Germany) or Sigma-Aldrich, Inc. (St. Louis, USA), unless stated otherwise. Absorbance and fluorescence measurements were conducted using the Infinite® M200 PRO Multimode Microplate Reader (Tecan Group, Männedorf, Switzerland). All biochemical analyses were performed in duplicate, and the results were standardized to milligram [mg] of total protein.

Pro-oxidant enzymes

The activity of erythrocyte NOX (EC 1.6.3.1) was determined by chemiluminescence, with lucigenin as the luminophore.[34] The rate of superoxide radical anion formation in the presence of NOX was measured. Enzymatic activity was defined as the quantity of the enzyme required to catalyze the synthesis of 1 nM of the superoxide radical anion per minute. In addition, the activity of erythrocyte XO (EC 1.17.3.2) was measured according to the method of Prajda and Weber.[35] This involved measuring uric acid formation from xanthine and quantifying the increase in absorbance at 290 nm. The activity of XO was defined as the amount of the enzyme required to release 1 μmol of uric acid per minute. The results were expressed in mU/mg of protein.

Antioxidant barrier

Total glutathione concentration was evaluated using the colorimetric method based on the enzymatic reaction between a chemically reduced form of NADPH, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), and glutathione reductase.[36] Absorbance was measured at a wavelength of 412 nm. The results were expressed in μmol/mg of total protein. To determine the concentration of oxidized glutathione (GSSG), the samples were thawed, neutralized to pH 6–7 with 1 M triethanolamine hydrochloride, and then incubated with 2-vinylpyridine to inhibit glutathione oxidation. Subsequently, the concentration of GSSG was measured colorimetrically, employing a method similar to that used for total glutathione determination,[36] and calculated using a calibration curve for GSSG solutions. The results were expressed in μmol/mg of total protein. The concentration of reduced glutathione (GSH) was calculated as the difference between total glutathione and GSSG concentration. The results were expressed in μmol/mg of total protein. The redox status was calculated as the ratio of $[GSH]^2$ to [GSSG].[37]

The total antioxidant status was determined with a colorimetric assay, using a commercial kit (Randox Laboratories, Crumlin, UK). The samples were incubated at 37°C with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), peroxidase (metmyoglobin) and H_2O_2 until the

formation of the $ABTS^{\bullet+}$ radical. The antioxidants present in the sample suppressed color production to a degree proportional to their concentration. The measurements were conducted at a wavelength of 660 nm.

Oxidative stress

The concentration of carbonyl groups (PC) was measured colorimetrically based on the reaction with 2,4-dinitrophenylhydrazine (DNPH).[38] The increase in the absorbance of the resulting hydrazone was measured at 360 nm. The concentration of PC was calculated using the molar absorption coefficient (ε) for DNPH, which was determined to be 22,000 $M^{-1}cm^{-1}$. The results were expressed in nmol/mg of protein.

Total thiol concentration was estimated colorimetrically according to Ellman's method.[39] This involved the utilization of DTNB, which was reduced to 2-nitro-5-thiobenzoic acid in the presence of thiol groups. The results were expressed in μmol/mg of protein.

The concentration of MDA was determined with a colorimetric assay, using thiobarbituric acid (TBA).[40] Malondialdehyde reacts with TBA to produce a colored adduct, with maximum absorbance at 535 nm. The concentration of MDA was measured in duplicate and expressed in mmol/mg of total protein.

The concentration of LOOHs was measured colorimetrically. In this approach, LOOH reacts with a ferrous iron to form a ferric iron, which then reacts with 3,32-bis[N,N-bis(carboxymethyl)aminomethyl]-o-cresolsulfonephthalein disodium salt (the xylenol orange reagent) to form a chromogen (the XOF complex).[40] The absorbance of XOF was measured at 560 nm. The results were expressed in μmol/mg of protein.

Glycoxidation products

The concentration of advanced glycation end-products (AGEs) was determined using a fluorescence-based assay according to the method described by Kalousová et al.[41] In this protocol, the fluorescence of furoyl-furanyl imidazole (FFI), carboxymethyl-lysine (CML), pyrraline, and pentosidine was measured at excitation and emission wavelengths of 350 nm and 440 nm, respectively. To determine the concentration of AGEs, the plasma samples were diluted 1:5 (v/v) in PBS (0.02 M, pH 7.0) and thoroughly mixed. Subsequently, a 200-microliter aliquot of the diluted sample was transferred to a 96-well microplate for fluorescence measurements.[42] The concentration of AGEs was determined in duplicate and expressed in arbitrary fluorescence units (AFU) per milligram of total protein.

To determine the content of amino acids modified during glycoxidation reactions (dityrosine, kynurenine, N-formylkynurenine, and tryptophan), the plasma samples were diluted 1:10 (v/v) in 0.1 M sulfuric acid and thoroughly mixed. A 200-microliter aliquot of the diluted sample was transferred to a 96-well microplate. Fluorescence was measured at various wavelength pairs: 330/415 nm (dityrosine), 365/480 nm (kynurenine), 325/434 nm (N-formylkynurenine), and 95/340 nm (tryptophan).[42,43] The content of amino acids modified by glycoxidation reactions was expressed in AFU/mg of total protein. All measurements were conducted in duplicate.

Nitrosative stress

The concentration of nitric oxide (NO) was determined with a colorimetric assay, as described by Grisham et al.[44] In this approach, nitrate (NO_3^-) reacts with sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride to produce a colored product, with maximum absorbance at 490 nm. The concentration of NO was calculated using a calibration curve for sodium nitrate ($NaNO_3$). The analyzed parameter was measured in duplicate and expressed in μmol/mg of total protein.

The concentration of S-nitrosothiols was determined with a colorimetric assay according to the protocol proposed by Wink et al.[45] In this approach, S-nitrosothiols present in the sample are quantified using the Griess reagent, following the reaction with the mercury ions (Hg^{2+}). The resulting compound exhibits maximum absorbance at 490 nm. The concentration of S-nitrosothiols was calculated using ε = 11,500 $M^{-1}cm^{-1}$. The analyzed parameter was measured in duplicate and expressed in μmol/mg of total protein.

The concentration of peroxynitrite was determined via a fluorometric assay by measuring the nitrosylation of phenol.[46] The reaction between peroxynitrite and phenol produces S-nitrophenol, which exhibits maximum absorbance at 490 nm (excitation) and 530 nm (emission). The concentration of peroxynitrite was calculated using ε = 1,670 $M^{-1}cm^{-1}$. The analyzed parameter was measured in duplicate and expressed in μmol/mg of total protein.

The concentration of nitrotyrosine was quantified using the Nitrotyrosine ELISA commercial kit (Immundiagnostik, Bensheim, Germany) according to the manufacturer's instructions. The results were expressed in nmol/mg of protein.

Pro-inflammatory and anti-inflammatory cytokines

The concentrations of IL-1β, IL-6, IL-8, and IL-10 were determined using commercial ELISA kits (EIAab Science, Wuhan, China) according to the manufacturer's instructions. Absorbance was measured at 405 nm, and the results were expressed in pg/mL.

Total protein concentration

Total protein concentration was determined with a colorimetric assay, using the commercial Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Rockford, USA),

based on bicinchoninic acid (BCA). Bicinchoninic acid reacts with the copper ions (Cu^{2+}) to form a stable complex, with maximum absorption at 562 nm.[47] Total protein concentration was read from a calibration curve for bovine serum albumin (BSA), and expressed in µg/mL.

Statistical analysis

The statistical analysis was conducted using the GraphPad Prism 9.5.1 software (GraphPad Software, La Jolla, USA). The normality of data distribution was checked with the Shapiro–Wilk test. The mean values for the experimental and control groups were compared using Student's *t* test at a significance level of $p \leq 0.05$. All data was presented as mean and standard deviation ($M \pm SD$). The number of subjects in each group was determined based on our previous experiment ($n = 15$), with the aim of achieving a test power of 0.8 (ClinCalc sample size calculator; https://clincalc.com/stats/samplesize.aspx).

Results

Routine laboratory tests

The results of the routine laboratory tests are shown in Table 1. In both the control and experimental groups, the values of all biomarkers were within the reference ranges. However, the levels of white blood cells (WBC), potassium ions (K^+) and C-reactive protein (CRP) were significantly higher in the experimental group as compared to the control group, while the hemoglobin (HGB), hematocrit (HCT) and platelet count (PLT) values were significantly lower.

Pro-oxidant enzymes

The activity of erythrocyte NOX (+19.12%; $p = 0.024$) and XO (+14.71%; $p = 0.045$) was significantly higher in maxillofacial surgery patients than in the control group (Fig. 1).

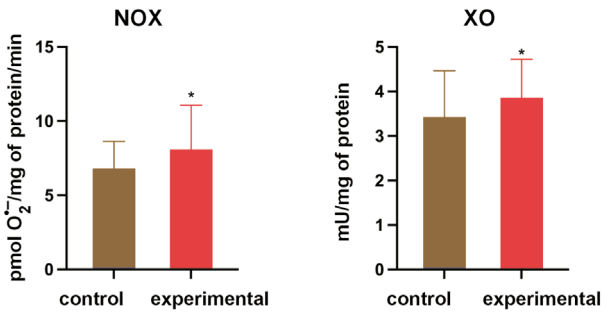

Fig. 1. Activity of pro-oxidant enzymes in the erythrocytes of control and experimental groups

NOX – nicotinamide adenine dinucleotide phosphate (NADPH) oxidase; XO – xanthine oxidase; *$p < 0.05$.

Table 1. Routine laboratory tests in the control and experimental group

Parameter	Control group ($n = 40$)	Experimental group ($n = 40$)	*p*-value	Reference value
WBC [$\times 10^3/\mu L$]	5.53 ±0.75	6.23 ±1.04	0.001*	4.0–10.0
RBC [$\times 10^6/\mu L$]	4.71 ±0.52	4.80 ±0.39	0.356	4.5–6.0
HGB [g/dL]	15.24 ±0.92	14.63 ±1.10	0.009*	14.0–18.0
HCT [%]	47.87 ±3.58	46.16 ±3.68	0.038*	40.0–54.0
MCV [fL]	85.64 ±4.30	85.38 ±3.72	0.773	80.0–94.0
MCHC [g/dL]	34.25 ±1.85	34.15 ±1.55	0.789	31.0–37.0
PLT [$\times 10^6/\mu L$]	260.40 ±36.90	232.70 ±53.67	0.009*	130.0–350.0
PT [s]	13.48 ±1.43	13.73 ±1.26	0.410	12.0–16.0
APTT [s]	29.50 ±4.37	28.97 ±3.48	0.550	26.0–40.0
INR	0.98 ±0.10	0.99 ±0.11	0.627	0.8–1.2
Na^+ [mmol/L]	139.20 ±2.55	139.00 ±2.85	0.680	136.0–145.0
K^+ [mmol/L]	4.14 ±0.46	4.40 ±0.56	0.029*	3.5–5.1
Glucose [mg/dL]	90.05 ±5.68	88.42 ±6.78	0.246	70.0–99.0
Creatinine [mg/dL]	0.96 ±0.15	0.90 ±0.12	0.053	0.7–1.2
Urea [mg/dL]	25.23 ±5.55	26.09 ±6.72	0.532	10.0–50.0
AST [U/L]	26.55 ±5.09	27.53 ±6.08	0.439	5.0–34.0
ALT [U/L]	28.98 ±6.16	28.30 ±8.92	0.695	0.0–55.0
CRP [mg/L]	1.61 ±0.55	3.07 ±1.33	0.0001*	0.0–10.0

Data presented as mean ± standard deviation ($M \pm SD$).
WBC – white blood cells; RBC – red blood cells; HGB – hemoglobin; HCT – hematocrit; MCV – mean corpuscular volume; MCHC – mean corpuscular hemoglobin concentration; PLT – platelet count; PT – prothrombin time; APTT – activated partial thromboplastin time; INR – international normalized ratio; Na^+ – sodium ions; K^+ – potassium ions; AST – aspartate transaminase; ALT – alanine transaminase; CRP – C-reactive protein; * statistically significant.

Antioxidant barrier

The total glutathione (–44.73%; $p < 0.0001$) and GSH (–44.73%; $p < 0.0001$) plasma levels were significantly lower in the experimental group than in the control group. The plasma concentration of GSSG was significantly higher in the experimental group than in the control group (+33.33%; $p < 0.0001$). Maxillofacial surgery patients exhibited significantly lower plasma TAS values in comparison with the control group (–9.80%; $p = 0.007$).

Furthermore, the redox status was significantly lower in the experimental group relative to the controls (–73.02%; $p < 0.0001$) (Fig. 2).

Oxidative stress

The plasma levels of MDA (+12.70%; $p = 0.035$) and LOOHs (+20.00%; $p < 0.0001$) were significantly higher in maxillofacial surgery patients in comparison with the control group. Additionally, the plasma concentration of PC was higher in the experimental group patients (+9.68%). However, the difference between the groups was not statistically significant. The plasma levels of total thiols were significantly lower in the experimental group than in the control group (–8.11%; $p = 0.025$) (Fig. 3).

Glycoxidation products

The plasma levels of dityrosine (+60.71%; $p < 0.0001$) and N-formylkynurenine (+7.14%; $p = 0.012$) were significantly higher in maxillofacial surgery patients in comparison with the control group. No significant differences were observed in the plasma concentrations of AGEs, kynurenine or tryptophan between the control and experimental group subjects (Fig. 4).

Nitrosative stress

The experimental group patients exhibited significantly higher plasma concentrations of NO (+10.26%; $p = 0.001$), S-nitrosothiols (+20.34%; $p = 0.002$), peroxynitrite

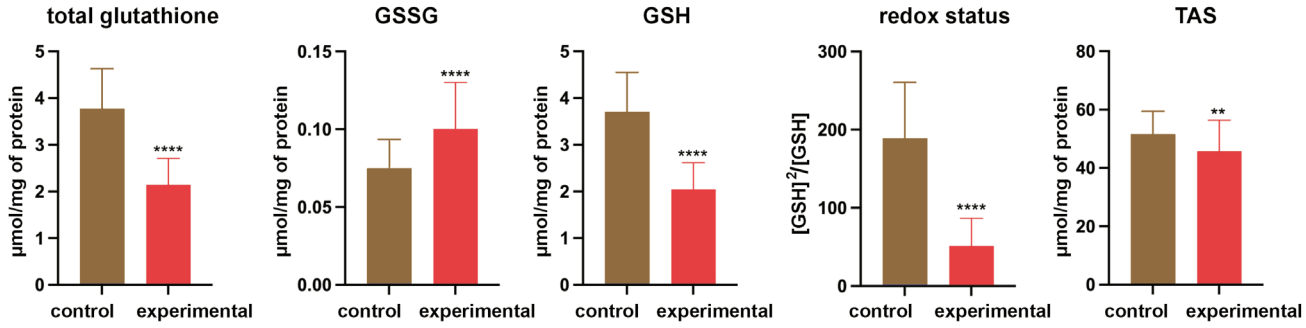

Fig. 2. Antioxidant barrier in the blood plasma of control and experimental groups
GSSG – oxidized glutathione; GSH – reduced glutathione; TAS – total antioxidant status; ** $p < 0.01$; **** $p < 0.0001$.

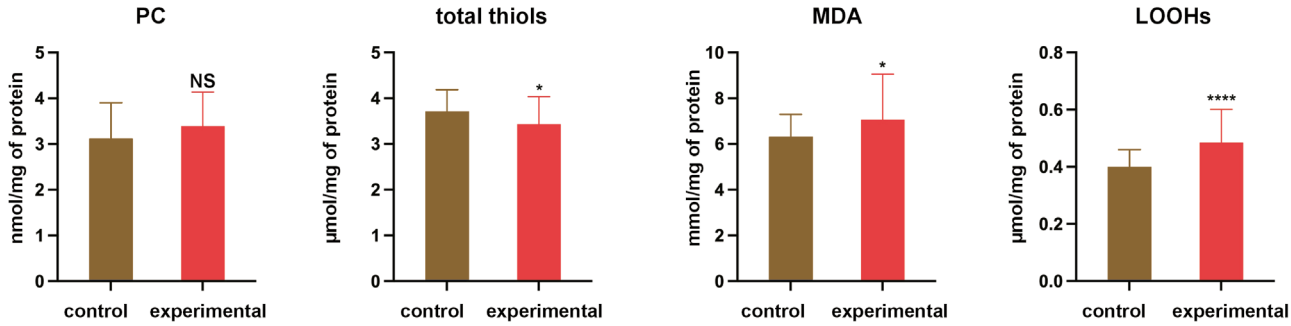

Fig. 3. Oxidative stress indicators in the blood plasma of control and experimental groups
PC – carbonyl groups; MDA – malondialdehyde; LOOHs – lipid hydroperoxides; * $p < 0.05$; **** $p < 0.0001$; NS – non-significant.

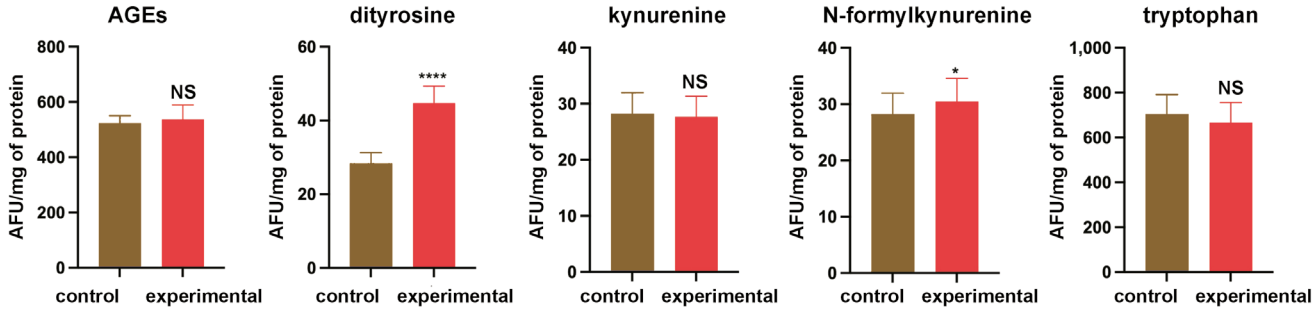

Fig. 4. Glycoxidation products in the blood plasma of control and experimental groups
AGEs – advanced glycation end-products; AFU – arbitrary fluorescence units; * $p < 0.05$; **** $p < 0.0001$; NS – non-significant.

(+10.53%; p = 0.006), and nitrotyrosine (+40.00%; p < 0.0001) as compared to the control group subjects (Fig. 5).

Pro-inflammatory and anti-inflammatory cytokines

The blood plasma levels of IL-1β (+5.61%; p = 0.005) and IL-6 (+5.68%; p = 0.009) were found to be significantly higher in maxillofacial surgery patients as compared to the control group subjects. No significant differences in the concentrations of IL-8 or IL-10 were observed between the control and experimental groups (Fig. 6).

ROC analysis

The receiver operating characteristics (ROC) curve is a graphic representation of the relationship between the sensitivity and specificity of a test. The ROC curve is used to evaluate the diagnostic accuracy of a biomarker. The area under the curve (AUC) measures the capacity of a test to discriminate between correct and incorrect results. A higher AUC indicates greater diagnostic accuracy for a given biomarker.

Among the analyzed biomarkers, total glutathione, GSH, the redox status, and dityrosine were identified as having the greatest potential significance for clinical application. These parameters demonstrated the highest sensitivity (≥85%) and specificity (≥85%) in discriminating between patients treated with Ti implants and control group subjects (AUC of 0.946, 0.948, 0.954, and 1.000, respectively) (Table 2).

Discussion

This study is the first to evaluate the circulating biomarkers of inflammation, protein glycoxidation and nitrosative damage in maxillofacial surgery patients treated with Ti fixations. An increase in the activity of pro-oxidant enzymes (↑ NOX, ↑ XO), impaired glutathione metabolism (↓ total glutathione, ↑ GSSG, ↓ GSH, ↓ redox status), higher levels of oxidative stress (↓ total thiols, ↑ MDA, ↑ LOOHs, ↑ TAS), carbonyl stress (↑ dityrosine, ↑ N-formylkynurenine) and nitrosative stress (↑ NO, ↑ S-nitrosothiols, ↑ peroxynitrite, ↑ nitrotyrosine), as well as an intensified systemic inflammatory response (↑ IL-1β, ↑ IL-6), were observed in maxillofacial surgery patients.

Some researchers have proposed that Ti implants should be removed from the mandible due to potential long-term adverse effects.[32,48] In the 1970s, Ti miniplates and screws were routinely removed, even if no side effects were reported.[49,50] Currently, Ti mandibular fixations are removed only when they induce inflammatory changes, become exposed, hinder prosthetic treatment, or cause subjective discomfort in patients, such as excessive

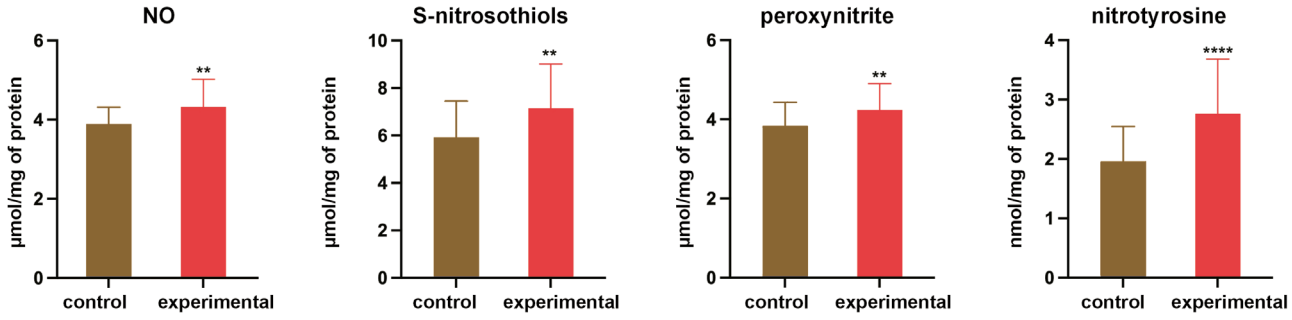

Fig. 5. Nitrosative stress indicators in the blood plasma of control and experimental groups
NO – nitric oxide; ** p < 0.01; **** p < 0.0001.

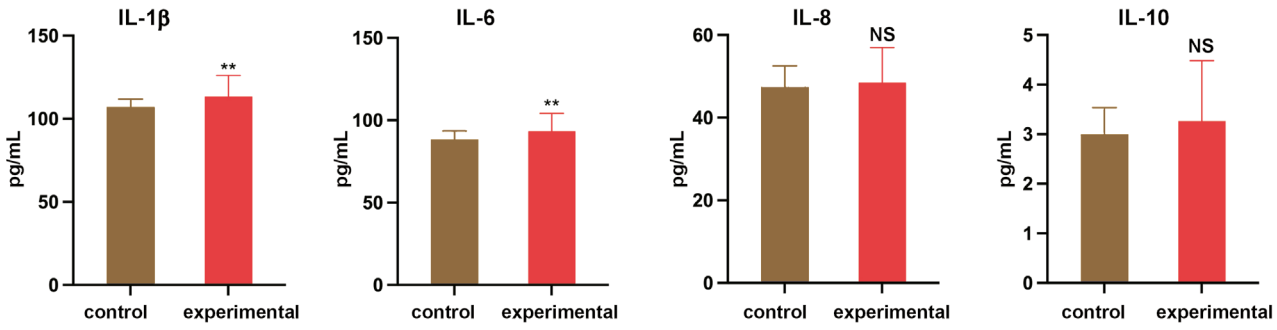

Fig. 6. Pro-inflammatory and anti-inflammatory cytokines in the blood plasma of control and experimental groups
IL – interleukin; ** p < 0.01; NS – non-significant.

Table 2. Diagnostic accuracy of the analyzed biomarkers

Category	Biomarker	AUC	*p*-value	Cut-off value	*CIs*	Sensitivity [%]	Specificity [%]
Pro-oxidant enzymes	NOX [pmol $O_2^{\bullet-}$/mg of protein/min]	0.647	0.028*	>7.082	0.5220–0.7718	60.0	62.5
	XO [mU/mg of protein]	0.629	0.046*	>3.736	0.5055–0.7532	62.5	65.0
Antioxidant barrier	total glutathione [µmol/mg of protein]	0.946	<0.0001*	<2.876	0.9014–0.9911	90.0	87.5
	GSSG [µmol/mg of protein]	0.755	<0.0001*	>0.083	0.6478–0.8622	67.5	67.5
	GSH [µmol/mg of protein]	0.948	<0.0001*	<2.783	0.9044–0.9919	90.0	87.5
	redox status $[GSH]^2/[GSSG]$	0.954	<0.0001*	<104.500	0.9150–0.9927	85.0	85.0
	TAS [µmol/mg of protein]	0.698	0.002*	<48.780	0.5805–0.8157	72.5	70.0
Oxidative stress	PC [nmol/mg of protein]	0.598	0.133	>3.220	0.4727–0.7223	52.5	55.0
	total thiols [µmol/mg of protein]	0.637	0.035*	<3.501	0.5131–0.7606	65.0	62.5
	MDA [mmol/mg of protein]	0.591	0.160	>6.534	0.4641–0.7184	57.5	55.0
	LOOHs [µmol/mg of protein]	0.753	<0.0001*	>0.437	0.6422–0.8628	67.5	70.0
Glycoxidation products	AGEs [AFU/mg of protein]	0.617	0.080	>528.600	0.4872–0.7403	57.5	55.0
	dityrosine [AFU/mg of protein]	1.000	<0.0001*	>35.350	1.0000–1.0000	97.5	100.0
	kynurenine [AFU/mg of protein]	0.539	0.545	<28.090	0.4122–0.6666	55.0	52.5
	N-formylkynurenine [AFU/mg of protein]	0.649	0.022*	>28.490	0.5284–0.7691	57.5	55.0
	tryptophan [AFU/mg of protein]	0.616	0.075	<680.900	0.4916–0.7396	55.0	57.5
Nitrosative stress	NO [µmol/mg of protein]	0.696	0.003*	>4.051	0.5780–0.8145	67.5	65.0
	S-nitrosothiols [µmol/mg of protein]	0.728	0.0004*	>6.298	0.6155–0.8408	70.0	67.5
	peroxynitrite [µmol/mg of protein]	0.691	0.003*	>4.019	0.5731–0.8094	67.5	65.0
	nitrotyrosine [nmol/mg of protein]	0.768	<0.0001*	>2.340	0.6621–0.8729	72.5	75.0
Pro-inflammatory and anti-inflammatory cytokines	IL-1β [pg/mL]	0.670	0.009*	>109.200	0.5414–0.7986	62.5	65.0
	IL-6 [pg/mL]	0.676	0.007*	>90.200	0.5557–0.7968	62.5	65.0
	IL-8 [pg/mL]	0.543	0.513	>47.540	0.4130–0.6720	50.0	47.5
	IL-10 [pg/mL]	0.546	0.476	>3.059	0.4132–0.6793	52.5	55.0

AUC – area under the curve; *CI* – confidence interval; * statistically significant.

sensitivity to cold at the implant site or discomfort upon palpation.[11,49,51] In the present study, Ti mandibular fixations were removed to eliminate the future risk of adverse reactions to a foreign body. Most importantly, none of the patients experienced any complications during the healing process. Inflammatory changes, reddening, swelling, abscesses, allergic reactions, or implant exposure could have affected the concentrations of the evaluated biomarkers. However, no such changes were observed in the peri-implant area.

In healthy individuals, the mechanisms of homeostatic control enable adaptation to variable environmental conditions. These mechanisms neutralize external factors that can potentially damage tissues and organs, triggering a local immune response, such as inflammation or oxidative stress. The type and intensity of the adaptive response are determined by the type of a foreign threat and the duration of exposure to that factor. It is believed that the adaptive response triggered by the immune system in the presence of a foreign body can proceed in stages over an extended period of time.

In the present study, systemic oxidative and nitrosative stress, as well as inflammation, were observed in patients treated with Ti mandibular fixations. However, these effects were not observed in the control group subjects. Although only the circulating biomarkers of redox balance and inflammation were evaluated, it can be assumed that systemic changes were induced by inflammation and oxidative stress at the local level. In our previous study, grayish discoloration of the peri-implant tissues was observed in patients undergoing bimaxillary osteotomy, confirming that implant surgery results in metallosis.[11] Although metal particles and ions can be released during the surgical placement of miniplates and screws, metallosis is primarily caused by implant surface wear that affects the surrounding tissues.[8,26] Interestingly, severe tribocorrosion (material degradation caused by the combined effect of corrosion and wear) was observed during the surgical fixation of the mandible, which is the only movable bone of the skull.[27,52] Mechanical wear is intensified in the presence of hard metallic debris, which has abrasive properties and leads to secondary wear. The metallic debris (Ti particles and nanoparticles) released at the peri-implant site can be phagocytized by the circulating immune cells (neutrophils, monocytes) and macrophages, which become stimulated to release pro-inflammatory cytokines (IL-1, IL-6, IL-8) and free radicals.[10,17,53] This process has been observed to have adverse effects on cells and tissues. The induction of the inflammatory response and ROS overproduction lead to the activation of fibroblasts and osteoclasts, the stimulation of the osteolysis processes,[54,55] the inhibition of type I collagen synthesis by osteoblasts,[56] and the production of Ti(IV)-specific T cells, which aggravate inflammation.[57] Titanium particles and ions can also contribute to genome instability, which is implicated in the initiation of carcinogenesis.[58]

In the present study, only patients without systemic diseases, infections or complications during the process of fracture healing were included in the experimental group, thereby supporting the hypothesis that inflammation was induced by a local immune response in the peri-implant tissues. However, despite a significant increase in the plasma levels of IL-1β and IL-6 in the experimental group, the leukocyte count and other routinely assessed inflammatory biomarkers (e.g., CRP) remained within the reference range. Furthermore, no significant differences in the plasma levels of IL-8 and IL-10 were observed between the 2 groups. These observations indicate the presence of mild systemic inflammation in patients with Ti implants, possibly attributable to the reduced activity of the immune system cells engaged in a local immune response. Reactive oxygen species are primarily generated by immune cells, which could also explain the mild symptoms of systemic oxidative/carbonyl stress.[59] Despite a significant increase in lipid peroxidation biomarkers (MDA and LOOHs), the concentrations of PC, AGEs, kynurenine, and tryptophan did not differ significantly between the experimental group and the control group. Lipids are the primary targets of ROS activity in cells. The process of lipid peroxidation is a chain reaction that results in the synthesis of secondary radicals, which then initiate subsequent reactions.[60]

In patients treated with Ti implants, a reduction in the plasma concentrations of total glutathione and GSH was observed, along with an increase in the levels of GSSG. Similar observations were made in previous studies, suggesting that GSH plays an important role in a local immune response in the peri-implant tissues of the mandibular periosteum.[26,61] Glutathione acts as the first line of defense against toxic metals. This antioxidant enhances mitochondrial activity and decreases ROS production, thus protecting tissues from oxidative stress.[26,62] Thiol groups also play a similar role, which explains the observed decrease in plasma thiol concentrations in the experimental group. The ROC analysis revealed that the redox status, and the plasma total glutathione, GSH and dityrosine levels were the most sensitive and specific biomarkers for discriminating between patients treated with Ti mandibular implants and control group subjects. Although further research is needed to validate this observation, the abovementioned parameters could be considered potential biomarkers for evaluating the adverse effects of Ti fixation devices.

Special attention should be paid to the increase in the concentrations of the circulating biomarkers of nitrosative stress (NO, S-nitrosothiols, peroxynitrite, nitrotyrosine). Previous research demonstrated that nitrosative stress could be induced by the macrophages activated by the circulating Ti particles, thereby increasing the expression of inducible NO synthase (iNOS).[63] Inducible NOS not only triggers NO overproduction for peroxynitrite synthesis, but also increases the synthesis of several pro-inflammatory mediators (IL-1β, IL-6).[64] At the local level, increased production of peroxynitrite disrupts metabolic processes and bone remodeling in the peri-implant area.[65] Although iNOS expression in blood cells is mainly determined by endothelium-dependent vasoconstriction, it appears that the circulating Ti particles may also increase the bioavailability of cytotoxic NO at the systemic level.

Strengths and limitations

The study had both strengths and limitations. One of its greatest strengths was the composition of the experimental group, with the subjects carefully selected to ensure equivalence with the control group based on the participants' age, general health and surgical procedures (including the number and quality of osteosynthesis implants). With regard to the main circulating biomarkers of inflammation and oxidative/nitrosative stress, the patients were observed for only 3–4 months after surgery, which represents a limitation of the study. Another issue is the fact that in patients with large joint (hip or knee) prostheses, immune-mediated inflammatory responses, as well as oxidative/nitrosative stress, are likely to be intensified, which makes them easier to observe. Finally, it is not possible to definitively determine whether the observed changes were caused by the influence of Ti or the surgical procedure itself. Therefore, further studies on the long-term effects of Ti implants are required. The timing of exposure is a key factor contributing to the adverse effects of Ti fixations. In the future, the possibility of generalizing the results should be enhanced by planning multicenter studies, or including larger and more diverse patient populations.

Conclusions

The present study showed that Ti mandibular fixations caused systemic oxidative/nitrosative stress and inflammation, whereas no such changes were observed in the control group. Although only the circulating biomarkers of redox balance and inflammation were evaluated, it is probable that systemic changes were induced by local immune responses. Patients treated with Ti mandibular implants exhibited mild symptoms of systemic inflammation and oxidative stress. Titanium miniplates and screws were removed 3–4 months after surgery, once full bone healing had been achieved. It is possible that the observed changes resulted from an early adaptive immune response to a foreign object in the body. Therefore, long-term research is needed to confirm or reject the hypothesis that Ti mandibular implants induce chronic inflammation and oxidative stress in patients. Interestingly, in patients treated for mandibular fractures, inflammatory changes at the Ti-6A1-4V implant site were observed several months or several years after osteosynthesis, which could be due to individual characteristics and the presence of comorbidities.

Ethics approval and consent to participate

The experiment was conducted in accordance with the Declaration of Helsinki (1964). The study protocol was approved by the Bioethics Committee of the Medical University of Bialystok, Poland (approval No. R-I-002/3/2-16). All patients were informed about the purpose of the study and the type of planned examinations, and they agreed to participate in the experiment by signing informed consent forms.

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.

ORCID iDs

Bożena Antonowicz https://orcid.org/0000-0001-8759-9260
Jan Borys https://orcid.org/0000-0002-5434-1631
Anna Zalewska https://orcid.org/0000-0003-4562-0951
Małgorzata Żendzian-Piotrowska https://orcid.org/0000-0002-4350-0369
Kamila Łukaszuk https://orcid.org/0000-0002-0095-6681
Łukasz Woźniak https://orcid.org/0000-0001-6889-1012
Mariusz Szuta https://orcid.org/0000-0002-7182-4811
Mateusz Maciejczyk https://orcid.org/0000-0001-5609-3187

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Original paper

Periodontal condition and quality of life in patients with controlled chronic systemic diseases: A cross-sectional study

Lourdes Zeballos Lopez[1,A,C,D,F], Lauro Taques Neto[1,A,C,D,F], Guilherme Arcaro[2,B,D,F], Fabiana Gabriel da Rosa[1,B,D,F], Kanandha Teixeira Cruz[1,B,D,F], Marcia Thais Pochapski[1,A,C,E,F], Fábio André dos Santos[1,A,C,E,F]

[1] Department of Dentistry, State University of Ponta Grossa, Brazil
[2] Department of Nursing and Public Health, State University of Ponta Grossa, Brazil

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):237–245

Address for correspondence
Fábio André dos Santos
E-mail: fasantos@uepg.br

Funding sources
The research was financially supported by the Coordination for the Improvement of Higher Education Personnel (CAPES), Ministry of Education, Brazil (code 001).

Conflict of interest
None declared

Acknowledgements
The authors would like to thank Dr. Sean J. Stroud for his thorough review of the manuscript and constructive feedback. The graphical abstract was created with BioRender.com.

Received on January 5, 2023
Reviewed on March 5, 2023
Accepted on March 9, 2023

Published online on April 23, 2025

Cite as
Lopez LZ, Taques Neto L, Arcaro G, et al. Periodontal condition and quality of life in patients with controlled chronic systemic diseases: A cross-sectional study. *Dent Med Probl.* 2025;62(2):237–245. doi:10.17219/dmp/162016

DOI
10.17219/dmp/162016

Abstract

Background. Chronic systemic diseases and periodontal diseases have an impact on an individual's quality of life. Both conditions exacerbate an individual's health status.

Objectives. The aim of the study was to examine whether periodontal condition could have an impact on the overall quality of life in patients with controlled chronic systemic diseases.

Material and methods. This cross-sectional study included 252 male and female subjects, aged ≥18 years, with a minimum of 6 teeth, and under medical follow-up for chronic systemic diseases. The following exclusion criteria were used: pregnant or lactating women; psychological or neurological limitations; uncontrolled chronic systemic disease; undergoing chemotherapy, radiotherapy, periodontal treatment, or tooth whitening; or use of an orthodontic appliance within the previous 3 months. The Medical Outcomes Study (MOS) 36-Item Short-Form Survey (SF-36) was used to assess the impact of periodontal health on patients' overall quality of life. To assess self-perception of periodontal condition, a self-reported periodontal disease measurement questionnaire was used. The periodontal assessment was performed by 2 calibrated dentists. Anamnesis forms were completed to collect sociodemographic, behavioral and medical diagnostic data, as well as to identify risk factors.

Results. The majority of the study participants were ≤50 years old (51%), female (65%), had a low education level (≤12 years of study) (60%), and resided in low-income households (93%). The study found no association between periodontal condition and quality of life. The majority of individuals with tooth mobility and 3–10 natural teeth were diagnosed with stage III and stage IV periodontitis. No significant relationship was identified between chronic systemic diseases and periodontitis.

Conclusions. Periodontal disease has been demonstrated to have no effect on the overall quality of life of individuals with controlled chronic systemic diseases. Self-reported cases of periodontal diseases corresponded with the clinical condition. Chronic systemic diseases were not identified as a risk factor for the development of periodontitis.

Keywords: quality of life, periodontitis, chronic disease, self-perception

Highlights

- Periodontal disease did not influence the overall quality of life in individuals with controlled chronic systemic diseases.
- There was no significant association between chronic systemic diseases and periodontitis.
- Self-reported periodontal symptoms corresponded closely with clinical periodontal diagnoses.
- Combined use of clinical assessment and self-report tools proved effective in identifying periodontal care needs.

Introduction

According to the Global Burden of Disease Study 2019 (GBD 2019), approx. 44% of the global population is affected by oral disorders such as dental caries, periodontitis and edentulism.[1] These conditions can cause disability, resulting in pain, sepsis, lost school days, decreased work productivity, and overall worse quality of life and well-being.[2] Periodontal disease is a multifactorial, chronic inflammatory disease induced by subgingival dental biofilm that causes the loss of tooth-supporting tissues. This can result in a systemic proinflammatory state, which is implicated in the etiology of various chronic diseases, including cardiovascular disease, diabetes, osteoporosis, mental health conditions, and autoimmune diseases.[3] Furthermore, the inflammatory mediators present in the gingival and peri-implant sulcus may contribute to the early diagnosis of periodontal and peri-implant diseases.[3,4]

The management of health-related habits that contribute to the development of chronic diseases has not improved, and the prevalence of multimorbidity is on the rise, affecting 65% of individuals aged ≥65 years.[5] Oral and systemic diseases have a large impact on the quality of life of individuals from all age groups.[6] These conditions can influence self-esteem, nutrition, the ability to eat, health, and can cause pain, anxiety and social privation.[5–7]

Therefore, patient medical history, in addition to epidemiological indicators, is critical for planning, organizing and monitoring health services.[8] The medical history of an individual is further enriched by the integration of their perceptions and social representations of oral and systemic health conditions, as reported by the individual.[9] For this purpose, a valid self-reported measurement of disease can serve as a cost-effective method to facilitate epidemiological studies that incorporate population surveillance.[10] Self-reporting of diseases consistent with clinical parameters can contribute to the prevention and early diagnosis of periodontal diseases, especially in individuals who require complex clinical care.[11,12] Some studies have demonstrated the effectiveness of self-assessment as a tool for evaluating periodontal status and many other conditions.[13] Previous data has shown that self-perception can have acceptable validity, ranging from moderate to high compared to clinical examination.

The present study aimed to analyze whether periodontal conditions have an influence on the overall quality of life of patients with controlled chronic systemic diseases. The secondary objectives were to verify the relationship between clinical periodontal diagnosis and self-perception of periodontal condition, and to assess whether chronic systemic diseases are risk factors for periodontal disease.

Material and methods

Study population

This cross-sectional study was designed according to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.[14] The Institutional Ethics Committee of the State University of Ponta Grossa, Brazil, has approved this study (protocol No. 3028211).

The research was conducted at the Regional University Hospital of Campos Gerais, State University of Ponta Grossa, in the southern region of Brazil, from December 2018 to May 2019. The sample size calculation was based on a previous study that used self-reporting questionnaires regarding periodontal disease.[15]

According to a self-report survey, 68% of the population have gingival problems.[15] To estimate this proportion with 5% absolute precision and 95% confidence, a minimum sample size of 246 volunteers is required from a potentially eligible population of 928 participants (Fig. 1). Based on the sample size calculator (https://www.statulator.com/SampleSize/ss1P.html), if a random sample of 246 is selected and 68% of subjects in a sample exhibit gingival problems, a confidence level of 95% can be ascribed to the hypothesis that between 63% and 73% of the subjects in the population possess the factor of interest.

Male and female subjects aged ≥18 years, with a minimum of 6 natural teeth, under medical follow-up for chronic systemic diseases, and without changes in medication during the previous 2 months were included in the study. The most prevalent systemic diseases were included (circulatory system diseases, nutritional, metabolic or endocrine diseases, respiratory system diseases, and immune system diseases). The following exclusion criteria were used: pregnant or lactating women; psychological or neurological limitations; uncontrolled chronic systemic

disease; undergoing chemotherapy, radiotherapy, periodontal treatment, or tooth whitening; or use of an orthodontic appliance within the previous 3 months (Fig. 1).

Data collection

An anamnesis form was completed based on patients' medical records to collect data on sociodemographics, behavioral habits, risk factors for periodontitis, current medication, and medical diagnosis. The patients were categorized into 4 disease groups according to the International Classification of Diseases (ICD-11),[16] as follows: group 1 (circulatory system diseases); group 2 (nutritional, metabolic or endocrine diseases); group 3 (respiratory system diseases); and group 4 (immune system diseases). The Medical Outcomes Study (MOS) 36-Item Short-Form Survey (SF-36) and a self-reported periodontal disease measurement questionnaire were subsequently administered to the patients. Then, the patients underwent a comprehensive clinical periodontal examination. Individuals in need of immediate treatment were referred to the university hospital's dental service.

Assessment of quality of life and self-reported periodontal condition

The instrument used to evaluate the impact of health on patients' overall quality of life was the SF-36 (Brazilian-Portuguese version). This questionnaire is composed of 36 items grouped into 8 subscales with multidimensional aspects, which evaluate the following: functional capacity; physical aspects; general health status; emotional aspects; social aspects; pain; vitality; and mental health. The SF-36 score ranges from 0 to 100, with 0 representing the worst state of health and 100 representing the best possible state.[17]

The patients' self-perception of their periodontal condition was assessed using the self-reported periodontal disease measurement questionnaire (Brazilian-Portuguese version),[18] which was based on self-reported questions from previous studies.[12,19] The questions were either objective or had a cognitive basis, prompting the individual to analyze their oral condition. The questionnaire contained 22 items on the following topics: 4 sociodemographic questions; 5 questions related to risk factors (such as smoking, diabetes and pregnancy); 10 self-reported questions concerning oral health and periodontitis; 2 questions on the history of periodontal treatment; and 1 question on the professional report of periodontal disease. The following variables were incorporated into the analysis: gingivitis; tooth migration; tooth mobility; tooth loss; number of natural teeth; oral health classification; scaling and root planing; periodontal surgery; and bone loss. Both questionnaires were previously validated and adapted for the Portuguese language.

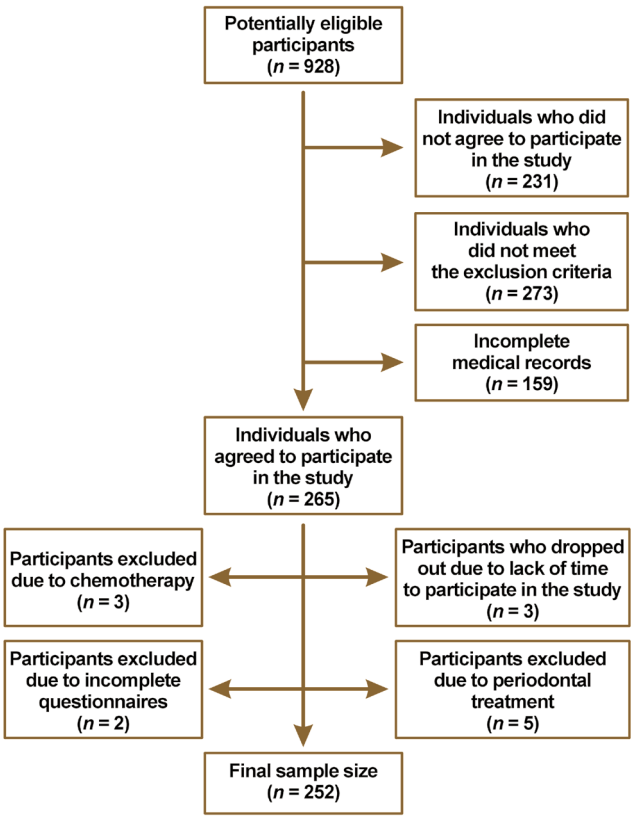

Fig. 1. Flowchart of the study

Clinical periodontal evaluation

The following dental and periodontal parameters were evaluated: missing teeth; marginal suppuration; dental biofilm; gingival recession; probing depth; bleeding on probing; and clinical attachment loss. Examinations were conducted using a manual Millenium Plus North Carolina CP-15 periodontal probe (Golgran, São Caetano do Sul, Brazil) that included all teeth at 6 sites per tooth, with the exception of third molars. The classification of periodontal disease for subsequent diagnosis was based on the criteria established in the report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions.[20] We categorized the patients into the following 4 groups: periodontal health or gingivitis (gingivitis was defined as bleeding on probing at ≥10% of the sites); stage I periodontitis (clinical attachment loss of 1–2 mm); stage II periodontitis (clinical attachment loss of 3–4 mm); and stage III and stage IV periodontitis (clinical attachment loss of ≥5 mm).

The periodontal evaluation was conducted by 2 trained and calibrated dentists (LZL and LTN). The training and calibration for assessing clinical parameters (dental biofilm, bleeding on probing and marginal suppuration) were carried out through discussions among the researchers during joint clinical examinations in the preliminary phase of the study. The inter-examiner reliability for gingival recession, probing depth and clinical attachment loss was determined using the weighted Cohen's kappa.

The inter-examiner agreement was substantial, with values of 0.74 for gingival recession, 0.92 for probing depth, and 0.85 for clinical attachment loss.

Statistical analysis

The χ^2 test was applied to evaluate the association between general population parameters according to age and sex. The same test was used to verify whether the self-perceived periodontal condition was associated with the periodontal diagnosis. We compared the quantitative periodontal parameters for age and sex using the unpaired *t*-test. Prior to conducting all analyses, the normality of the data was tested. To ascertain the impact of periodontal condition on patients' overall quality of life, the analysis of variance (ANOVA) was applied in conjunction with Tukey's post hoc test. The binary logistic regression model was used to determine whether controlled chronic systemic diseases function as risk factors for periodontal disease. The model incorporated all disease groups as predictor variables. The dichotomous response variable was the presence or absence of periodontitis, and the data was reported as odds ratios (*OR*s) and 95% confidence intervals (*CI*s). The results were considered statistically significant for $p < 0.05$.

Results

Characteristics of the population

The total number of subjects included in the study was 252. The majority of the participants were ≤50 years old (51%), female (65%), had received a maximum of 12 years of education (60%), and resided in low-income households (93% with incomes up to $700.00).

Behavioral habits and oral hygiene

All the variables were analyzed based on sex and age of the participants. The majority of smokers were ≤50 years of age, while ex-smokers were predominantly ≥51 years of age ($p = 0.001$). In addition, ex-smokers were more frequently male ($p = 0.014$). With respect to diabetes, no association was identified between age and sex. Patients aged ≥51 years consumed more medications per day ($p = 0.002$). There was a significant association between the variables of flossing and frequency of flossing in relation to age and sex regarding oral hygiene habits. The use of dental floss was more prevalent among female subjects ($p = 0.003$) and among individuals aged ≤50 years ($p = 0.006$).

Table 1. Behavioral habits and oral hygiene of the study participants ($N = 252$)

Variable		Age		*p*-value	Sex		*p*-value
		≤50 years ($n = 128$)	≥51 years ($n = 124$)		female ($n = 164$)	male ($n = 88$)	
Smoking status	non-smoker	87 (68)	77 (62)	0.001*	116 (71)	48 (55)	0.014*
	smoker	20 (16)	6 (5)		17 (10)	9 (10)	
	ex-smoker	21 (16)	41 (33)		31 (19)	31 (35)	
Smoking time	<10 years	8 (40)	0 (0)	0.063	6 (35)	2 (22)	0.492
	≥10 years	12 (60)	6 (100)		11 (65)	7 (78)	
Cigarettes/day, *n*	<10	10 (50)	5 (83)	0.147	11 (65)	4 (44)	0.320
	≥10	10 (50)	1 (17)		6 (35)	5 (56)	
Ex-smoking time	<10 years	10 (48)	16 (39)	0.516	15 (48)	11 (35)	0.303
	≥10 years	11 (52)	25 (61)		16 (52)	20 (65)	
Diabetes	no	96 (75)	84 (68)	0.202	117 (71)	63 (72)	0.967
	yes	32 (25)	40 (32)		47 (29)	25 (28)	
Medications/day, *n*	none	27 (21)	16 (13)	0.002*	28 (17)	15 (17)	0.514
	1–3	61 (48)	42 (34)		71 (43)	32 (36)	
	4 and more	40 (31)	66 (53)		65 (40)	41 (47)	
Dental flossing	no	43 (34)	63 (51)	0.006*	58 (35)	48 (55)	0.003*
	yes	85 (66)	61 (49)		106 (65)	40 (45)	
Frequency of dental flossing	every day	39 (30)	63 (51)	0.016*	58 (35)	48 (54)	0.013*
	no use	43 (34)	32 (26)		52 (32)	19 (22)	
	1–3 times/week or other	46 (36)	29 (23)		54 (33)	21 (24)	
Brushing frequency	up to twice/day	48 (38)	53 (43)	0.396	51 (31)	50 (57)	<0.0001*
	3 or more times/day	80 (62)	71 (57)		113 (69)	38 (43)	

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

Daily dental flossing, however, was more prevalent among males ($p = 0.013$) and patients aged ≥51 years ($p = 0.016$). Women exhibited a higher frequency of toothbrushing than men, with daily toothbrushing significantly associated with female sex ($p < 0.0001$) (Table 1).

Periodontal conditions

The majority of individuals aged ≤50 years exhibited an average of 20–25 teeth and a lower tooth loss index in comparison to the subjects aged ≥51 years ($p < 0.0001$). A higher number of sites with biofilm ($p = 0.027$) and a greater clinical attachment loss of 3–4 mm or ≥5 mm ($p < 0.0001$) was observed in males aged ≥51 years compared to other groups. The prevalence of probing depth of 1–3 mm was higher among women ($p = 0.001$), whereas men exhibited a higher number of sites with probing depth of 4–5 mm ($p = 0.001$) and ≥6 mm ($p = 0.005$). The group with a mean age of ≤50 years presented a higher percentage of sites with bleeding on probing ($p = 0.001$) and a lower percentage of sites with gingival recession ($p < 0.0001$). A lower percentage of women showed sites with gingival recession (1–3 mm: $p = 0.002$; ≥4 mm: $p < 0.0001$) (Table 2).

Impact of periodontal condition on quality of life

The results regarding the quality of life subscale of SF-36 were similar among patients with different periodontal diagnoses ($p > 0.05$) (Table 3). The coefficients of variation between SF-36 subscales according to periodontal condition were low, supporting the similarity between the groups, as follows: functional capacity (3.4%); physical aspects (3.4%); pain (3.3%); general health status (0.4%); vitality (1.5%); social aspects (0.6%); emotional aspects (3.4%); and mental health (1.8%).

Self-reported periodontal disease measurement and clinical periodontal diagnosis

Individuals who reported having tooth mobility, tooth loss, scaling and root planing, and 3–10 natural teeth were mostly associated with stages III and IV periodontitis. A statistically significant association was identified between the stages of periodontitis and these variables ($p < 0.05$) (Table 4).

Table 2. Periodontal parameters of the study participants ($N = 252$)

Variable		Age		*p*-value	Sex		*p*-value
		≤50 years ($n = 128$)	≥51 years ($n = 124$)		female ($n = 164$)	male ($n = 88$)	
Teeth†, *n*		21.6 ±6.3	14.8 ±6.6	<0.0001*	18.3 ±7.5	18.4 ±7.0	0.912
Teeth‡, *n*	6–10	11 (9)	46 (37)	<0.0001*	39 (24)	18 (20)	0.738
	11–19	23 (18)	44 (35)		40 (24)	27 (31)	
	20–25	50 (39)	26 (21)		50 (30)	26 (30)	
	26–28	44 (34)	8 (6)		35 (21)	17 (19)	
Dental biofilm† [%]		68 ±25	72 ±26	0.261	67 ±25	75 ±26	0.027*
Bleeding on probing† [%]		52 ±22	43 ±22	0.001*	49 ±22	45 ±23	0.172
Marginal suppuration† [%]		3 ±8	5 ±13	0.632	4 ±10	5 ±12	0.391
Gingival recession† [%]	0 mm	92 ±13	73 ±24	<0.0001*	86 ±20	76 ±23	<0.0001*
	1–3 mm	6 ±10	19 ±16	<0.0001*	11 ±14	16 ±14	0.002*
	≥4 mm	1 ±3	8 ±13	<0.0001*	3 ±8	7 ±12	<0.0001*
Probing depth† [%]	1–3 mm	86 ±16	88 ±13	0.608	90 ±11	82 ±19	0.001*
	4–5 mm	12 ±14	11 ±11	0.701	10 ±10	16 ±16	0.001*
	≥6 mm	1 ±4	1 ±2	0.265	1 ±2	2 ±5	0.005*
Clinical attachment loss† [%]	0 mm	80 ±21	64 ±24	<0.0001*	77 ±22	63 ±25	<0.0001*
	1–2 mm	11 ±12	8 ±8	0.046*	8 ±9	12 ±12	0.027*
	3–4 mm	3 ±4	9 ±8	<0.0001*	5 ±7	7 ±7	0.018*
	≥5 mm	6 ±12	19 ±21	<0.0001*	9 ±16	18 ±2	<0.0001*

* statistically significant ($p < 0.05$); †*t*-test; ‡χ^2 test. Data presented as mean ± standard deviation (*M* ±*SD*) or as *n* (%).

Table 3. Results of the Medical Outcomes Study (MOS) 36-Item Short-Form Health Survey (SF-36) according to the periodontal diagnosis

SF-36 subscale	Periodontal health or gingivitis	Periodontitis			*p*-value (ANOVA)
		stage I	stage II	stages III and IV	
Functional capacity	50.7 ±9.6	51.8 ±10.3	51.4 ±9.1	48.0 ±10.5	0.155
Physical aspects	52.0 ±10.2	50.7 ±10.0	51.0 ±10.2	48.0 ±9.6	0.121
Pain	48.6 ±9.4	52.4 ±9.8	51.1 ±9.9	49.6 ±10.5	0.332
General health status	50.1 ±9.9	50.5 ±9.4	50.0 ±9.8	50.1 ±10.5	0.670
Vitality	49.5 ±10.8	49.8 ±11.2	51.2 ±9.0	49.9 ±9.6	0.720
Social aspects	50.5 ±10.5	49.8 ±10.0	50.0 ±9.5	49.9 ±10.1	0.987
Emotional aspects	51.3 ±9.9	51.5 ±10.0	47.8 ±10.2	49.9 ±9.9	0.333
Mental health	48.9 ±10.8	49.4 ±10.1	50.4 ±9.2	50.8 ±10.1	0.775

Data presented as *M* ±*SD*. The SF-36 score ranges from 0 to 100, with 0 representing the worst state of health and 100 representing the best possible state. Gingivitis was defined as bleeding on probing at ≥10% of the sites, stage I periodontitis as clinical attachment loss of 1–2 mm, stage II periodontitis as clinical attachment loss of 3–4 mm, and stages III and IV periodontitis as clinical attachment loss of ≥5 mm.

Table 4. Self-reported periodontal health and clinical periodontal diagnosis of the study participants

Variable		Patients (*N* = 252)	Periodontal health or gingivitis (*n* = 67)	Periodontitis			*p*-value
				stage I (*n* = 33)	stage II (*n* = 54)	stages III and IV (*n* = 98)	
Gingivitis	no	150 (60)	40 (60)	19 (58)	34 (63)	57 (58)	0.942
	yes	102 (40)	27 (50)	14 (42)	20 (37)	41 (42)	
Tooth migration	no	167 (66)	50 (75)	25 (76)	36 (67)	56 (57)	0.069
	yes	85 (34)	17 (25)	8 (24)	18 (33)	42 (43)	
Tooth mobility	no	185 (73)	60 (90)	21 (64)	43 (80)	61 (62)	0.001*
	yes	67 (27)	7 (10)	12 (36)	11 (20)	37 (38)	
Tooth loss	no	213 (85)	62 (93)	27 (82)	48 (89)	76 (78)	0.048*
	yes	39 (15)	5 (7)	6 (18)	6 (11)	22 (22)	
Natural teeth, *n*	3–10	64 (25)	17 (25)	4 (12)	12 (22)	31 (32)	0.002*
	11–19	53 (21)	7 (10)	7 (21)	13 (24)	26 (27)	
	20–27	79 (31)	17 (25)	13 (39)	22 (41)	27 (28)	
	≥28	56 (22)	26 (39)	9 (27)	7 (13)	14 (14)	
Oral health	excellent to good	131 (52)	40 (60)	15 (45)	25 (46)	51 (52)	0.413
	bad to very bad	121 (48)	27 (40)	18 (55)	29 (54)	47 (48)	
Scaling and root planing	no	175 (69)	51 (76)	24 (73)	43 (80)	57 (58)	0.019*
	yes	77 (31)	16 (24)	9 (27)	11 (20)	41 (42)	
Periodontal surgery	no	235 (93)	65 (97)	31 (94)	52 (96)	87 (89)	0.142
	yes	17 (7)	2 (3)	2 (6)	2 (4)	11 (11)	
Bone loss	no	222 (88)	63 (94)	31 (94)	46 (85)	82 (84)	0.134
	yes	30 (12)	4 (6)	2 (6)	8 (15)	16 (16)	

* statistically significant ($p < 0.05$, χ^2 test). Data presented as *n* (%). Gingivitis was defined as bleeding on probing at ≥10% of the sites, stage I periodontitis as clinical attachment loss of 1–2 mm, stage II periodontitis as clinical attachment loss of 3–4 mm, and stages III and IV periodontitis as clinical attachment loss of ≥5 mm.

Systemic condition and periodontal diagnosis

The predictors were previously defined as follows: circulatory system diseases (group 1); nutritional, metabolic or endocrine diseases (group 2); respiratory system diseases (group 3); and immune system diseases (group 4). When considered as a whole, these factors were not found to be associated with the risk of periodontitis ($p = 0.791$). After adjustment, the logistic regression analysis did not demonstrate a significant relationship between the risk of periodontitis and the defined predictor variables (Table 5).

Table 5. Logistic regression analysis of the impact of explanatory variables (i.e., chronic systemic diseases) on the risk of periodontitis

Variable	β	*SE*	Wald	*df*	*p*-value	*OR*	95% *CI* for *OR*
Group 1	0.29	0.55	0.28	1	0.596	1.34	0.46–3.91
Group 2	0.59	0.68	0.76	1	0.382	1.81	0.48–6.81
Group 3	0.12	0.69	0.03	1	0.864	1.13	0.29–4.31
Group 4	–0.29	0.55	0.28	1	0.596	0.75	0.26–2.19
Constant	1.99	0.44	20.96	–	0.001*	7.33	–

* statistically significant ($p < 0.05$); group 1 – circulatory system diseases; group 2 – nutritional, metabolic or endocrine diseases; group 3 – respiratory system diseases; group 4 – immune system diseases; *SE* – standard error; *df* – degrees of freedom; *OR* – odds ratio; *CI* – confidence interval.

Discussion

The findings of this study indicated that the quality of life did not vary according to the periodontal condition of individuals with controlled chronic systemic diseases. The quality of life subscales of the SF-36 demonstrated minimal variation between the patient groups. Although many studies have suggested that periodontal disease has a negative impact on the quality of life of individuals, these studies used specific questionnaires that predominantly addressed oral health aspects rather than generic questionnaires, such as the SF-36, that are widely used in patients with systemic diseases.[21–23] Our observations revealed no impact of periodontal disease on the overall quality of life when using a generic assessment instrument (SF-36). The findings of this study differ from those reported in the literature, possibly due to the fact that the patients included in our research had controlled systemic conditions. Additionally, this discrepancy may be attributed to other criteria used to classify periodontal diseases.[17,23]

In the current study, the majority of subjects who completed the self-reported periodontal assessment exhibited advanced stages of periodontal disease and a low family income. These factors could be associated with challenges in accessing healthcare services, which may have contributed to the observed prevalence and severity of periodontal disease.[24] The self-reported periodontal condition coincided with the clinical diagnosis.[18] Previous studies have attempted to determine the predictive capacity of self-reported periodontal disease measurement variables.[13] Although this tool does not estimate the severity of periodontal disease, it has been demonstrated to be effective in the rapid identification of periodontitis due to its non-invasiveness and cost-effectiveness.[25] However, the final diagnosis of the severity of periodontal disease must be made through a clinical examination by a qualified professional.

In the present study, chronic systemic diseases were not identified as risk predictors for the development of periodontitis. It should be emphasized that all subjects included in the study were under continuous medical supervision, with their chronic systemic diseases being adequately managed. This factor may explain the absence of associations between variables, a phenomenon that has been previously documented in related studies.[26,27]

The present study evaluated a vulnerable population group (systemic involvement, low income and low levels of education). The use of a clinical method for periodontal assessment, in conjunction with a self-reported periodontal disease questionnaire, enabled the identification of subjects requiring both clinical and informational intervention, as many exhibited little knowledge about the disease.[11–13,18] The individuals adequately self-reported their symptoms yet demonstrated no self-perception of the disease, perhaps due to poor understanding or a focus on medical monitoring for systemic conditions.[18,19,25]

Our study was subject to certain limitations, including the inability to measure incidence or establish causal relationships due to its cross-sectional design, the potential difficulty in interpreting identified associations, and the inability to investigate temporal relations between outcomes and risk factors. Additionally, the unequal distribution of sex among study participants and the use of a generic instrument to assess quality of life may have constrained the generalizability of the findings.[28]

The patients included in the present study had various systemic diseases, with multiple conditions frequently co-occurring. In addition, they used numerous medications to manage these diseases. Both systemic conditions and medications can cause alterations within the oral cavity, affecting the periodontal tissues and, by extension, the quality of life.[7,29] Therefore, the results of the study may have been influenced by these factors. However, we also identified positive and differentiated aspects, such as the use of 2 methods for evaluating periodontal conditions: the clinical assessment method; and the periodontal disease self-perception questionnaire, an instrument employed in large epidemiological studies. Despite its generic nature, the utilization of the SF-36 to assess quality of life enabled the analysis of functional capacity and subjective well-being in relation to the health status of the study participants.[17] Furthermore, to the best of the authors' knowledge, no previous studies have used the SF-36 to assess the impact of periodontal disease on the overall quality of life of patients with chronic systemic diseases.

The present research offers further insights into the existing literature on this subject, corroborating the idea that self-reported periodontal conditions may serve as

indicators of clinical periodontal status.[30] Conversely, the findings indicate that periodontal condition does not interfere with the quality of life as measured by a generic assessment instrument (SF-36). This suggests that chronic systemic diseases may have an impact on the physical and mental well-being of individuals. In addition, the outcomes of this study enabled the verification of patients' levels of knowledge and self-perceptions of periodontal disease. This verified information can facilitate the development of educational policies that encourage the prevention and early diagnosis of this disease.

Conclusions

Based on a sample of patients with systemic diseases who receive ongoing medical care and monitoring at a referral hospital, it can be concluded that periodontal disease did not interfere with the overall quality of life of individuals with controlled chronic systemic diseases. The final diagnosis of the severity of periodontal diseases must be made through a clinical examination by a qualified professional. Controlled chronic systemic diseases were not identified as a risk factor for the development of periodontitis. It is recommended that long-term follow-up studies be conducted to evaluate the impact of periodontitis on quality of life and the association between periodontitis and systemic diseases.

Ethics approval and consent to participate

The Institutional Ethics Committee of the State University of Ponta Grossa, Brazil, has approved this study (protocol No. 3028211).

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Lourdes Zeballos Lopez https://orcid.org/0000-0003-0451-8010
Lauro Taques Neto https://orcid.org/0000-0001-5686-9475
Guilherme Arcaro https://orcid.org/0000-0003-1855-9091
Fabiana Gabriel da Rosa https://orcid.org/0000-0002-4560-8656
Kanandha Teixeira Cruz https://orcid.org/0000-0002-2266-3468
Marcia Thais Pochapski https://orcid.org/0000-0003-4220-7838
Fábio André dos Santos https://orcid.org/0000-0003-0347-0270

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Original paper

Influence of the pharmacotherapy of general diseases on the severity of symptoms of temporomandibular disorders

Katarzyna Grad[A,C,D], Zuzanna Kazibudzka[A,C,D], Małgorzata Pihut[B,E], Aneta Wieczorek[E,F]

Department of Prosthodontics and Orthodontics, Dental Institute, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):247–253

Address for correspondence
Katarzyna Grad
E-mail: katarzynagrad09@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on August 4, 2023
Reviewed on October 23, 2023
Accepted on November 17, 2023

Published online on April 30, 2025

Abstract

Background. Temporomandibular disorders (TMD) constitute a heterogeneous group of conditions affecting the temporomandibular joints (TMJs) and the surrounding tissues. The etiology of these anomalies has been extensively discussed due to their multifactorial and diverse nature; however the influence of many factors remains ambiguous. Temporomandibular disorders may be manifested as pain or in a painless form, characterized by acoustic symptoms in TMJs or their dysfunction. The presence of a causal relationship between the use of medications for chronic diseases and TMD symptoms can simplify the diagnostic and therapeutic approach in TMD treatment.

Objectives. The aim of the study was to verify the existence of and assess the correlation between the usage of pharmacotherapy in chronic diseases and the occurrence and severity of TMD symptoms.

Material and methods. This retrospective study was based on the analysis of 252 questionnaires completed by patients who had previously reported to the University Dental Clinic in Krakow, Poland, due to the occurrence of TMD symptoms. The patients were categorized into 4 subgroups, depending on the type of drugs taken: endocrine; cardiological; psychotropic; and other. Data was subjected to statistical analysis.

Results. In the tested group, an association between the usage of endocrine drugs and the risk of headaches was observed. The patients taking cardiological drugs exhibited a reduced likelihood of experiencing difficulties in opening the mouth wide as compared to those under treatment for other reasons. However, no significant impact of the drugs on the intensity of TMD pain symptoms was observed. Furthermore, no correlation was found between the medications taken and the occurrence of clicking in TMJ and behaviors in the form of clenching and/or grinding of the teeth.

Conclusions. The pharmacotherapy of chronic diseases in TMD patients might be associated with an increased risk of headaches. Nonetheless, there were no statistically significant differences between the types of drugs taken with regard to the intensity of TMD pain symptoms, as well as the presence of clicking and behaviors in the form of clenching and/or grinding of the teeth.

Keywords: pharmacotherapy, temporomandibular disorders, TMD, general diseases

Cite as
Grad K, Kazibudzka Z, Pihut M, Wieczorek A. Influence of the pharmacotherapy of general diseases on the severity of symptoms of temporomandibular disorders. *Dent Med Probl.* 2025;62(2):247–253. doi:10.17219/dmp/175417

DOI
10.17219/dmp/175417

Highlights

- The use of endocrine drugs was identified as a factor associated with an increased risk of experiencing headaches.
- Among patients taking cardiological drugs, a reduced likelihood of difficulty in opening the mouth wide – a common symptom of temporomandibular disorders (TMD) – was observed as compared to those using medications from other drug categories.
- However, the use of endocrine, cardiological, psychotropic, and other medications showed no significant effect on the intensity of TMD pain symptoms in the studied population.

Introduction

Temporomandibular disorders (TMD) are a heterogeneous group of disorders concerning temporomandibular joints (TMJs) and the surrounding structures.[1] The prevalence of TMD in the general population is estimated at around 40%.[1] In young adults, the prevalence of TMD symptoms varies from 43% up to 60%.[1] The prevalence of TMD in children and adolescents varies from 16% to 68%.[2] They are the 2nd most common cause of craniofacial pain.[1,3–6] The peak incidence is between the age of 20 and 40. It is more common in women than in men, with a male-to-female ratio ranging from 1:2 in the general population to as high as 1:8 clinically,[3,7,8] especially in the reproductive age.[8,9] Not only the frequency, but also a higher severity of symptoms is correlated with female gender.[10] The prospective OPPERA (Orofacial Pain: Prospective Evaluation and Risk Assessment) study showed that an average of 4 new cases of TMD per 100 people develop each year.[11]

Due to their multifactorial nature, the etiology of TMD is still not fully elucidated. Modulators can be biological (e.g., internal derangements in TMJ), environmental, social (e.g., a learned response to pain), emotional, and cognitive elements (e.g., depression, anxiety and stress).[1,3,6] A significant influence of genetic, inflammatory and psychoemotional determinants has been proven.[12]

The OPPERA study clearly showed that poor general health increases the risk of developing TMD.[11] Patients with a subjective sense of impaired health are more likely to suffer from TMD symptoms. Temporomandibular disorders often coexist with other medical conditions. A significant correlation has been shown for headaches, including migraine, and psychoemotional disorders, in particular depression. Rheumatic joint diseases may also affect TMJs or predispose to their dysfunction.[10] A cross-sectional study conducted in Korea showed a high level of comorbidity of TMD with asthma, migraine, osteoarthritis, and thyroid disorders.[13] The painful form of TMD is often correlated with the presence of the coexisting ache in other areas of the body, such as chronic back pain, abdominal pain, migraine, and others.[14]

The increase in muscle tone, which is one of the causes of TMD, is influenced, among others, by systemic factors, i.e., hormonal disorders, thyroid diseases, metabolic disorders, migraine, immunological diseases, rheumatoid and degenerative diseases, genetic factors, and many others.[3]

Symptoms of TMD can be either painful or painless.[15] The painful form is characterized by the presence of craniofacial complaints; it affects TMJs, the masticatory muscles, and the head and neck muscles. Typical symptoms include TMJ pain (limited or abnormal abduction of the jaw), and ear, head or face pain. Symptoms can range from mild discomfort to debilitating pain, limiting the jaw function.[3] Patients most commonly report feeling tense, spontaneous pain at rest, or muscle ache associated with chewing. The painless symptoms of TMJ dysfunction, occurring locally, are primarily increased masticatory muscle tone, a feeling of muscle stiffness and numbness, the asymmetry of their action, and paresthesia in trigger points. The disorder of the mandible function, especially its limited and incorrect abduction, leads to the unsynchronized work of both TMJs. Reported complaints include clicking in TMJ, which occurs during mandibular abduction and adduction, as well as during protrusion and lateral movements. In advanced stages, some patients complain of the deterioration of their general mental and physical condition as a result of nagging headaches, sleep disorders or poor movement coordination.[15]

Patients most often seek help because of pain. Excessive muscle tension is predominantly associated with increased stress and subsequent behaviors that allow to relieve emotional tension, such as the clenching and/or grinding of the teeth.[12,16] According to the OPPERA data, the pain intensity level of new TMD cases was rated as mild or very mild.[11] The average pain intensity in the TMD population on a scale of 1–10 was 4.6.[7] Pain amplification may be modulated by genotypic and phenotypic factors.[7,8] The gender factor is an important determinant – clinical craniofacial pain symptoms are more common in women, they last longer and are more severe.[7,8] Women are also more likely to experience comorbid and multitarget pain.[17] It is important to notice that the occurrence of general health conditions also exhibits variability within the population. According to the Migraine in Poland study, headaches tend to occur more often in women (87.1%) than in men.[18]

Standardizing diagnostic and therapeutic procedures, and creating protocols of medical action are important from the perspective of combating such a complex phenomenon as TMD.[19]

The existence of numerous presumptions about the impact of general health and the medications taken on the occurrence of TMD was the motivation to conduct our research. The objective of the present study was to verify and assess the correlation between the medications taken for diagnosed chronic diseases and the occurrence and intensity of pain symptoms of TMD among patients admitted to the dental clinic. Establishing specific correlations can be very helpful in clinical practice. Conducting a clinical examination, supported by a thorough medical history could facilitate diagnosis in such cases, and consequently enable the implementation of effective treatment to alleviate the symptoms.[19] The presence of a causal relationship between the use of medications for chronic diseases and TMD symptoms can simplify the diagnostic and therapeutic approach in TMD treatment. To the best of our knowledge, no comprehensive study investigating this issue has been conducted so far. Therefore, we aimed to investigate whether the aforementioned correlation exists.

Material and methods

Participants

The study was retrospective and consisted in the verification of the questionnaires completed by patients who had previously reported to the University Dental Clinic in Krakow, Poland, due to alleged TMD in the years 2016–2019.

The study involved individuals who presented at the dental clinic with symptoms related to the stomatognathic system, suggestive of TMD. The data was collected through a proprietary questionnaire, which included questions about general health condition and the most frequent symptoms associated with TMD. Each patient received the questionnaire to complete as part of the initial assessment during their first visit.

After analyzing the completed questionnaire and conducting a clinical examination based on the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD),[20,21] the attending dentist made a diagnosis and established the appropriate treatment. In a situation when a patient reported 2 or more TMD symptoms, each of them was considered separately in relation to the medications taken for comorbid conditions.

The inclusion criteria for the study comprised the presence of at least one TMD symptom. Incomplete questionnaires were excluded from the study.

The analysis included 252 patients; 2 questionnaires were rejected due to incorrect answers to the questions.

Questionnaire

The questionnaire consisted of a part concerning personal data (name, surname, age), a medical history (presence of general diseases, medications taken), and a section on TMD and related symptoms, which included the following issues:

1. What is the reason for reporting to the clinic?
2. What medications are taken regularly?
3. How severe is your current masticatory muscle pain on a scale from 1 to 10?
4. What is the severity of your current TMJ pain on a scale from 1 to 10?
5. What was the average intensity of masticatory muscle pain in the last 8 weeks?
6. What was the average intensity of TMJ pain in the last 8 weeks?
7. Have you ever had a problem with opening your mouth wide?
8. Have you experienced clicks in TMJ? If so, for how long?
9. Are there any other additional symptoms?
10. Do you have headaches; what is their intensity and frequency?
11. Are there tooth clenching and/or grinding behaviors observed?

Statistical analysis

A multivariate analysis of the effect of different types of drugs on the quantitative variable was performed by linear regression. The results are presented as the regression model parameter values with a 95% confidence interval (*CI*). A multivariate analysis of the effect of different types of drugs on the dichotomous variable was performed by logistic regression. The results are presented as the odds ratio (*OR*) values with a 95% *CI*. A significance level of 0.05 was adopted in the analysis, so all *p*-values below 0.05 were interpreted as statistically significant. The analysis was performed in the R program, v. 4.2.2 (https://www.r-project.org/).

Results

The study included 252 questionnaires. The majority of the respondents were women (76%), while men accounted for 24%. Age ranged from 18 to 42 years. Among the reasons for reporting to the clinic, TMJ pain was the most common cause (n = 150; 60%), followed by clicking in TMJ (n = 126; 50%) and limited mouth opening (n = 60; 24%).

The analysis of the questionnaires made it possible to distinguish 4 groups of drugs taken by the patients: endocrine (24% of patients); cardiological (11% of patients); psychotropic (7% of patients); and other (18% of patients).

The emphasis was placed on the medications taken for chronic medical conditions, whereas analgesic medications were classified under the category of 'other medications' administered by the patients. The group of endocrine drugs administered by the patients included preparations used for thyroid function disorders (Levothyroxinum natricum) (46%) and contraceptives (23%).

Pain in the masticatory muscles

There was no statistically significant relationship between the drugs taken and the intensity of masticatory muscle pain in the study group (Tables 1 and 2).

Temporomandibular joint pain

Statistical analysis showed that the intensity of TMJ pain was not affected by taking endocrine, cardiological, psychotropic, or other drugs (Tables 3 and 4).

Headaches

The results of the analysis show that endocrine drugs are a significant independent predictor of the chance of headache occurrence, and the risk increases by 2.243 times. There was no statistically significant effect of taking cardiological, psychotropic and other drugs on the incidence of headaches (Table 5).

Problem with opening the mouth wide

An important independent predictor of the chance of having a problem with opening the mouth wide is taking cardiological drugs. In this group of patients, an 85.2% reduction in the chance of developing ailments was observed (Table 6).

Table 1. Influence of factors on the current intensity of masticatory muscle pain (a multivariate linear regression model)

Variable	Regression parameter	95% *CI*		*p*-value
		lower bound	upper bound	
Endocrine drugs	–0.184	–1.071	0.703	0.685
Cardiological drugs	0.315	–0.909	1.539	0.614
Psychotropic drugs	0.745	–0.764	2.254	0.334
Other drugs	0.683	–0.295	1.662	0.172

CI – confidence interval.

Table 2. Influence of factors on the average severity of masticatory muscle pain in the last 8 weeks (a multivariate linear regression model)

Variable	Regression parameter	95% *CI*		*p*-value
		lower bound	upper bound	
Endocrine drugs	0.013	–0.864	0.890	0.977
Cardiological drugs	0.424	–0.785	1.634	0.492
Psychotropic drugs	0.408	–1.084	1.900	0.592
Other drugs	0.567	–0.401	1.534	0.252

Table 3. Influence of factors on the current intensity of temporomandibular joint (TMJ) pain (a multivariate linear regression model)

Variable	Regression parameter	95% *CI*		*p*-value
		lower bound	upper bound	
Endocrine drugs	–0.184	–1.071	0.703	0.685
Cardiological drugs	0.315	–0.909	1.539	0.614
Psychotropic drugs	0.745	–0.764	2.254	0.334
Other drugs	0.683	–0.295	1.662	0.172

Table 4. Influence of factors on the average severity of temporomandibular joint (TMJ) pain in the last 8 weeks (a multivariate linear regression model)

Variable	Regression parameter	95% *CI*		*p*-value
		lower bound	upper bound	
Endocrine drugs	–0.157	–1.042	0.729	0.729
Cardiological drugs	0.054	–1.168	1.276	0.931
Psychotropic drugs	1.023	–0.484	2.53	0.185
Other drugs	0.746	–0.231	1.723	0.136

Table 5. Influence of factors on headache occurrence (a multivariate logistic regression model)

Variable	*OR*	95% *CI*		*p*-value
		lower bound	upper bound	
Endocrine drugs	2.243	1.184	4.251	0.013*
Cardiological drugs	1.319	0.555	3.136	0.531
Psychotropic drugs	2.516	0.782	8.092	0.122
Other drugs	1.796	0.892	3.615	0.101

OR – odds ratio; * statistically significant.

Table 6. Influence of factors on the chance of having a problem with opening the mouth wide (a multivariate logistic regression model)

Variable	*OR*	95% *CI*		*p*-value
		lower bound	upper bound	
Endocrine drugs	1.671	0.893	3.127	0.109
Cardiological drugs	0.148	0.053	0.414	<0.001*
Psychotropic drugs	2.481	0.792	7.770	0.119
Other drugs	1.402	0.711	2.765	0.330

* statistically significant.

Clicking

Taking endocrine, cardiological, psychotropic, and other drugs did not affect the occurrence of clicks.

Tooth clenching and/or grinding

There was no correlation between the intake of any of the tested groups of drugs and the occurrence of behaviors in the form of tooth clenching and/or grinding.

Discussion

The aim of the present study was to verify the existence of and assess the correlation between the applied general pharmacotherapy and the occurrence and intensity of subjective symptoms of TMD. Considering the study results, no connection between the use of drugs and the intensification of pain in TMJ can be found. The study showed that taking endocrine drugs increased the likelihood of headaches. No correlation was observed between the intake of any of the tested groups of drugs and the occurrence of clicks and behaviors in the form of clenching and/or grinding of the teeth. The patients taking heart medications had a lower chance of having a problem with opening the mouth wide as compared to those taking other classes of drugs.

Numerous studies have been conducted to determine the impact of various disease states on the occurrence of TMD. Hormones, as active substances, have a great impact on the body. Both thyroid hormones and female sex hormones have been proven to exert a significant influence on the metabolism of the tissues of the musculoskeletal and nervous systems, and pain conduction.

Thyroid hormones

Thyroid diseases are the most common endocrinopathies; they can affect even 2–5% of the population.[22] Their incidence increases with age, concerning approx. 15% of the population aged 75 years or older.[22] Thyroid diseases are much more common in women than in men.[23]

Thyroid hormones affect muscle contractility and metabolism. The main target of their signaling is the skeletal muscle, as exemplified by the myopathic symptoms observed in many patients with the thyroid dysfunction.[24] Triiodothyronine (T3) is involved in the embryonic and subsequent development of the skeletal muscle by stimulating the growth of fibers. Hypothyroidism is manifested by delayed muscle contraction and relaxation, whereas hyperthyroidism is characterized by excessive muscle contraction.[22,25,26]

The painless forms of TMD include primarily increased masticatory muscle tone, muscle stiffness, the asymmetry of their functioning, and paresthesia. The forms of pain are pressure soreness and the presence of trigger points. The manifestation of thyroid diseases in the musculoskeletal system suggests that they may affect the occurrence and symptoms of the masticatory system dysfunction.[22]

Studies have reported that there is a clear correlation between the incidence of TMD, especially muscular disorders, and the number of patients with Hashimoto's thyroiditis.[25] The relationship between the occurrence of thyroid diseases and TMJ pain regards about 70% of patients.[22]

Hypothyroidism is often accompanied by musculoskeletal symptoms ranging from muscle and joint pain to true myopathy and osteoarthritis. It has been speculated that hypothyroidism may manifest itself in the musculoskeletal system in the form of TMD.[27]

Estrogens

Female sex hormones – estrogens – have a great impact on the functioning of the human body. Numerous studies have been conducted to clearly determine the cause of TMD sex determination, but this dependency is very complex. Since the discovery of estrogen receptors in TMJ in 1986, the potential influence of these hormones on the occurrence and course of TMD has been studied. In addition, they are also present in the nervous system, e.g., in the nucleus of the trigeminal nerve. The impact of estrogens varies greatly, depending on the type of signal and the current conditions, i.e., the presence of inflammation and its type; therefore it is difficult to define it unequivocally.[9,28]

Various studies point to several conclusions:

– TMD incidence peaks at childbearing age[28];
– the greatest intensity of pain in women of reproductive age occurs in the perimenstrual period, and if they take contraceptives, also during ovulation[29];
– the use of contraceptives or hormone replacement therapy (HRT) is positively correlated with the occurrence of TMD, and the severity of symptoms increases with an increasing hormone dose[28];
– women with TMD during pregnancy, when there is a significant increase in the estrogen levels, experience a decrease in pain symptoms as compared to non-pregnant women of the same age[30–32];
– estrogen deficiency leads to subsequent structural alterations in the tissues of TMJ, which can lead to degenerative changes.[9]

Menopausal women are more likely than men of similar age to develop TMJ disease and age-related loss of the alveolar bone.[32]

Circulatory system

Contrary to the results of the current study, a clinical study conducted among children with cardiovascular diseases (CVD) showed a higher incidence of TMD in these patients than in healthy children, and it concerned acoustic symptoms.[33] Notwithstanding, the accompanying pain was milder in the CVD group as compared to controls. The overall occurrence of parafunctional behaviors in both groups did not differ; however, a higher incidence of bruxism was confirmed in children with CVD. The study showed no significant impact of the presence of the main disease on the coexistence of TMD pain or the severity of symptoms.[33]

Psychoemotional condition

One of the important etiological factors contributing to the development of TMD are psychoemotional disorders,

which include depression, dysthymia, panic attacks, anxiety states and neuroses, personality disorders, and sleep disorders.[12,34–36] Among the global population, 47% of adults suffer from headaches in general, 10% from migraine, 38% from tension-type headaches, and 3% from chronic headaches that last for more than 15 days per month.[5] Temporomandibular disorders decrease patients' quality of life (QoL), potentially limit their daily activities due to pain intensity and pain-related disability, and increase anxiety and depression.[6] Chronic stress is a factor that adversely affects the functioning of the stomatognathic system, especially the masticatory muscles.[12,37] An increased level of emotional stress generates behaviors in the form of clenching and/or grinding of the teeth, which are very harmful to the masticatory system.[12]

Antidepressants administered to patients at risk of depression and/or migraine may cause headaches. Although some patients report clear benefits in terms of headache relief with the use of selective serotonin reuptake inhibitors (SSRIs), there have also been numerous reports of headache or migraine worsening as a result of SSRI use. On the other hand, serotonin-norepinephrine reuptake inhibitors (SNRIs) and tricyclic antidepressants (TCAs) appear to have the opposite effect, potentially alleviating headaches.[38]

The potential role of cardiovascular medications in reducing headaches among individuals with migraine is currently under investigation. The first-line medications in this category include beta blockers without intrinsic sympathomimetic activity (such as atenolol, bisoprolol, metoprolol, or propranolol), as well as topiramate and candesartan.[39]

General medical data on past and ongoing general diseases and pharmacotherapy should be collected from the patient during the first visit, before taking further diagnostic and therapeutic steps. Determining the relationship between the occurrence of specific general disorders or the preparations used and the development and severity of TMD symptoms can significantly facilitate the diagnostic and therapeutic processes with regard to TMD.

There is no clear information in the literature confirming the influence of thyroid, heart and other general diseases on the development of TMD symptoms. It is necessary to conduct in-depth research in this field to determine the relationship between the considered phenomena.

Limitations

The main limitation of this study is its retrospective nature, which does not allow gathering more detailed information from patients; only the data collected in the past can be used. In addition, there was no thorough distinction between the drugs taken and the diagnosed general diseases; their duration and course, and the stage of drug application were not determined as well. The authors did not utilize a validated questionnaire in the study, which represents another limitation. Therefore, to ensure the reproducibility of results, it is recommended to employ a validated questionnaire in future studies. Another constraint is the lack of reference to gender and age in the statistical analysis.

Conclusions

There was no impact of endocrine, cardiological, psychotropic, and other drugs on the intensity of pain symptoms of TMD in the examined population, both currently experienced and their average value from the 2 months preceding the visit.

Taking endocrine drugs is a factor that increases the risk of headaches.

In the patients taking cardiological drugs, a decrease in the chance of having a problem with opening the mouth wide was observed as compared to those taking drugs from other groups.

No correlation was observed between the intake of any of the tested groups of drugs and the occurrence of clicks and behaviors in the form of clenching and/or grinding of the teeth.

Further exploration of these phenomena is needed to establish clear dependencies between systemic diseases and the occurrence of TMD.

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.

ORCID iDs

Katarzyna Grad https://orcid.org/0009-0005-7926-2672
Zuzanna Kazibudzka https://orcid.org/0000-0002-3374-5231
Małgorzata Pihut https://orcid.org/0000-0002-0239-4328
Aneta Wieczorek https://orcid.org/0000-0003-2506-7394

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Original paper

Comparison of facial soft tissue thickness in subjects with different malocclusions according to the lateral cephalograms of an Iranian adult population

Milad Ali Asgharlou[1,A,D], Mahsa Esfehani[2,A,B,E], Maryam Tofangchiha[3,B,C], Amir Javadi[4,C], Nima Sheikhdavoodi[5,B,C], Zahra Yousefi[3,E], Rodolfo Reda[6,A,B,E], Luca Testarelli[6,B,F]

[1] Student Research Committee, Qazvin University of Medical Sciences, Iran
[2] Department of Oral and Maxillofacial Medicine, School of Dentistry, Qazvin University of Medical Sciences, Iran
[3] Department of Oral and Maxillofacial Radiology, Dental Caries Prevention Research Center, Qazvin University of Medical Sciences, Iran
[4] Department of Social Medicine, Qazvin University of Medical Sciences, Iran
[5] Department of Orthodontics, Qazvin University of Medical Sciences, Iran
[6] Department of Oral and Maxillofacial Sciences, Sapienza University of Rome, Italy

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):255–263

Address for correspondence
Maryam Tofangchiha
E-mail: mt_tofangchiha@yahoo.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on April 17, 2023
Reviewed on June 8, 2023
Accepted on August 23, 2023

Published online on April 30, 2025

Cite as
Asgharlou MA, Esfehani M, Tofangchiha M, et al. Comparison of facial soft tissue thickness in subjects with different malocclusions according to the lateral cephalograms of an Iranian adult population. *Dent Med Probl.* 2025;62(2):255–263. doi:10.17219/dmp/171501

DOI
10.17219/dmp/171501

Abstract

Background. Facial characteristics are determined by the thickness of facial soft tissue and parameters of the dentoskeletal system.

Objectives. The aim of the study was to compare the soft tissue thickness in individuals with different malocclusions, based on their lateral cephalograms.

Material and methods. In this retrospective study, 285 lateral cephalograms of 141 males and 144 females aged between 18 and 40 years were evaluated in the following 4 groups of malocclusions: class I ($n = 72$); class II division 1 ($n = 71$); class II division 2 ($n = 72$); and class III ($n = 70$). Linear measurements of the soft tissue thickness were obtained at 9 facial midline landmarks. Angular measurements were made by calculating the ANB angle and the inclination angle of upper central incisors. The data was statistically analyzed using the χ^2 test, the Shapiro-Wilk test, the *t*-test, analysis of variance (ANOVA), Tukey's test, and Pearson's correlation.

Results. A comparison of the soft tissue thickness revealed that male subjects demonstrated greater thickness than female subjects. This difference was statistically significant at all points in the skeletal class III individuals ($p < 0.05$). Different skeletal classes demonstrated significant differences in the soft tissue thickness at the subnasale, stomion, labrale inferius, labiomentale, and menton ($p < 0.05$).

Conclusions. Males exhibited a greater facial soft tissue thickness than females. A statistically significant difference in the soft tissue thickness was observed among the different skeletal classes, particularly at regions located farther from the underlying bone. The class III individuals revealed an increased upper lip thickness and a decreased lower lip thickness. A decrease in the upper lip thickness and an increase in the lower lip thickness were observed in the class II individuals. This pattern suggests that the underlying skeletal discrepancy is being concealed.

Keywords: malocclusion, soft tissue, cephalometry, thickness

Highlights

- Males have thicker facial soft tissue than females, with noticeable differences in soft tissue thickness.
- Significant differences in facial soft tissue thickness were observed across various skeletal classes.
- Individuals with class III skeletal malocclusion exhibited increased upper lip thickness and decreased lower lip soft tissue thickness.
- In contrast, class II skeletal individuals showed reduced upper lip thickness and increased lower lip thickness.

Introduction

In orthodontics, patients are typically classified into 3 distinct categories (class I, II and III) based on the skeletal relationship between the maxilla and mandible.[1] Discrepancies in this relationship can lead to a convex or concave facial profile. The facial profile does not always correspond to the skeletal profile due to variations in the soft tissue thickness among individuals. The assessment of facial appearance and profile must take into account the soft tissue and its impact on facial appearance. Thus, it is imperative to identify the standard pattern of the soft tissue thickness in different skeletal classes for each population, taking into account variations in anthropometric indices.[2]

As the importance of facial profile in orthodontic treatment has increased, the gold standard of orthodontic treatment results has undergone a gradual shift from hard tissue standards to soft tissue norms. Thus, finding the standard pattern and paradigm of the soft tissue thickness in different populations and racial groups can be of great significance.[3,4]

Several studies worldwide have attempted to obtain a specific paradigm regarding the mean soft tissue thickness in different populations. This subject has been previously explored by Perović and Blažej[4] in Serbia and Sarilita et al.[5] in Indonesia. However, to date, no studies on the topic have been conducted on the Iranian population. A comprehensive understanding of soft tissue thickness patterns across different skeletal classes would facilitate enhanced aesthetic outcomes for orthodontists.

Objectives

Orthodontists unanimously agree that facial aesthetics, oral function, and the mobility of the jaws and teeth are primarily influenced by the soft tissue thickness. Altered soft tissue thickness can directly affect the treatment plan and selection of surgical or non-surgical, and extraction or non-extraction orthodontic treatment plans. Additionally, the soft tissue thickness is important in the camouflage orthodontic treatment of many patients. The understanding of the precise pattern of the soft tissue thickness is crucial in facial reconstructions, particularly in relation to the remaining hard tissue. This knowledge could also aid in forensic medicine investigations. In face-driven orthodontics, the interplay between the face and the temporomandibular joint (TMJ) is instrumental in achieving optimal orthodontic outcomes. In consideration of the aforementioned factors, the objective of this study was to compare the soft tissue thickness in individuals with different malocclusions as determined by lateral cephalograms.

Material and methods

Study design

This retrospective study was conducted on 285 lateral cephalograms of 141 males and 144 females retrieved from the archives of a maxillofacial radiology center at Parto Maxillofacial Specialty Clinic, Qazvin, Iran. The study was approved by the Ethics Committee of Qazvin University of Medical Sciences, Iran (IR.QUMS.REC.1401.197).

Linear measurements of the soft tissue thickness were made at 9 facial midline landmarks. Angular measurements were obtained by calculating the ANB angle and the inclination angle of upper central incisors. The mean thickness of the facial soft tissue was evaluated for each group.

Setting

All lateral cephalograms were obtained using the Cranex® 3D X-ray system (Soredex, Tuusula, Finland), which utilizes a protective filter with a thickness equivalent to 2.7 mm of aluminum. The exposure settings were 73 kV, 10 mA, and a 165-cm distance from the tube. The patient's head was in its natural position and fixed by the cephalostat. The tube exhibited a 90° angle relative to the sagittal plane, and the Frankfurt plane was parallel to the horizon. The lips were relaxed, while the teeth demonstrated centric occlusion. All patients underwent a precise radiographic evaluation, and their skeletal and soft tissue profiles were analyzed using lateral cephalograms.

Participants

The inclusion criteria were lateral cephalograms of males and females aged 18–40 years, and availability of complete demographic information of participants. The lateral cephalograms had been obtained for purposes not related to this study, such as orthodontic treatment planning. The exclusion criteria encompassed developmental and congenital anomalies, mixed dentition, a history of maxillofacial trauma, a history of orthodontic treatment or cosmetic procedures, and cephalograms with distortion.

Variables

The lateral cephalograms were traced, and 9 hard tissue anatomical landmarks and their corresponding soft tissue analogs were identified according to the literature.[1,4–10] The landmarks were as follows:

– G–G'-1: glabella region, defined as the linear distance between point G (most prominent point of the frontal bone) and its corresponding soft tissue analog point (Fig. 1A);
– N–N': nasion region, defined as the linear distance between point N and its soft tissue analog point (at the fusion of frontal and nasal bones) (Fig. 1B);
– A–Sn: subnasale region, defined as the distance between point A (most concave point of the maxilla) and the subnasale (Sn) (Fig. 1C);
– Pr–Ls: distance between the prosthion (Pr) and the upper lip surface (labrale superius (Ls)) (Fig. 1D);
– J–St: distance between point J (most labial point of upper incisors) and the contact point of the upper and lower lips (stomion (St)) (Fig. 1E);
– Id–Li: distance between the infradentale (Id) (most anterior and superior point on the alveolar ridge between lower central incisors) and the lower lip surface (labrale inferius (Li)) (Fig. 1F);
– B–B': distance between point B (most concave point of the mandibular symphysis) and point B' (labiomentale or the most concave point of the soft tissue of mandibular symphysis) (Fig. 1G);
– Pog–Pog': distance between the pogonion (Pog) or most prominent point of the chin and its soft tissue analog point (Fig. 1H);
– Me–Me': distance between the most inferior point of the mandible (menton (Me)) and its soft tissue analog point (Fig. 1I).

The schematic view of facial landmarks is outlined in Fig. 2.

Data sources and measurement

The ANB angle was traced and measured in accordance with the American Board of Orthodontics (ABO) analysis. Subsequently, AudaxCeph software, v. 6.1.4.395

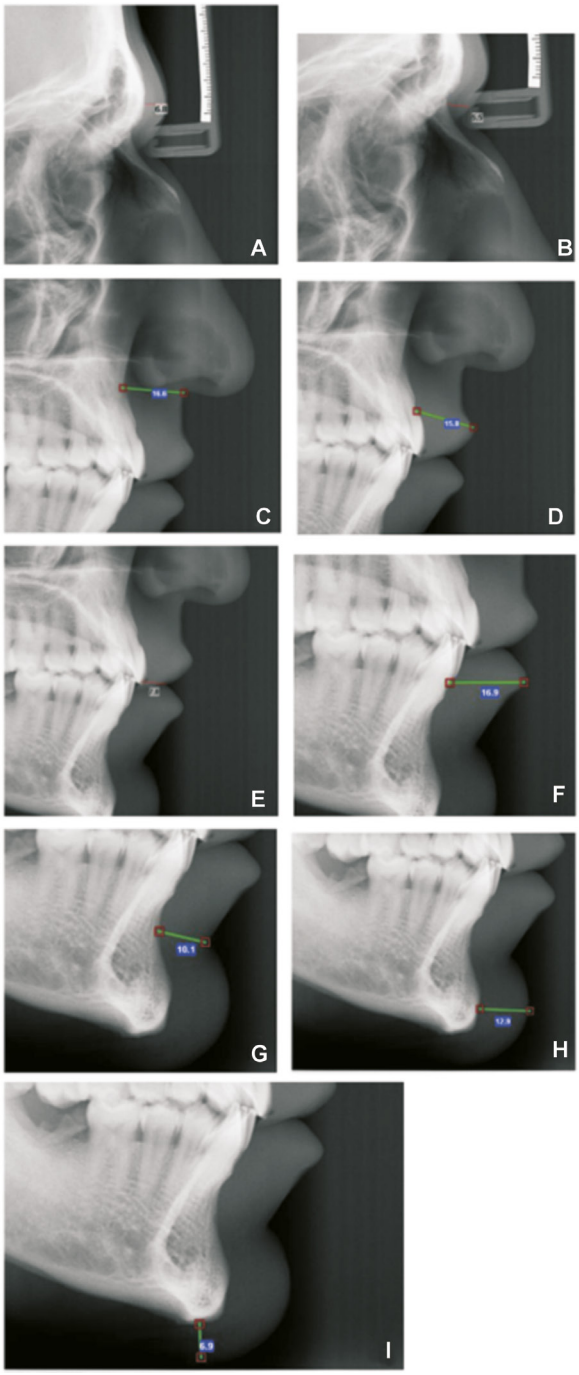

Fig. 1. Cephalometric linear measurements

A. G–G'-1 – glabella region, defined as the linear distance between point G (most prominent point of the frontal bone) and its corresponding soft tissue analog point; B. N–N' – nasion region, defined as the linear distance between point N and its soft tissue analog point (at the fusion of frontal and nasal bones); C. A–Sn – subnasale region, defined as the distance between point A (most concave point of the maxilla) and the subnasale (Sn); D. Pr–Ls – distance between the prosthion (Pr) and the upper lip surface (labrale superius (Ls)); E. J–St – distance between point J (most labial point of upper incisors) and the contact point of the upper and lower lips (stomion (St)); F. Id–Li – distance between the infradentale (Id) (most anterior and superior point on the alveolar ridge between lower central incisors) and the lower lip surface (labrale inferius (Li)); G. B–B' – distance between point B (most concave point of the mandibular symphysis) and point B' (labiomentale or the most concave point of the soft tissue of mandibular symphysis); H. Pog–Pog' – distance between the pogonion (Pog) or most prominent point of the chin and its soft tissue analog point; I. Me–Me' – distance between the most inferior point of the mandible (menton (Me)) and its soft tissue analog point.

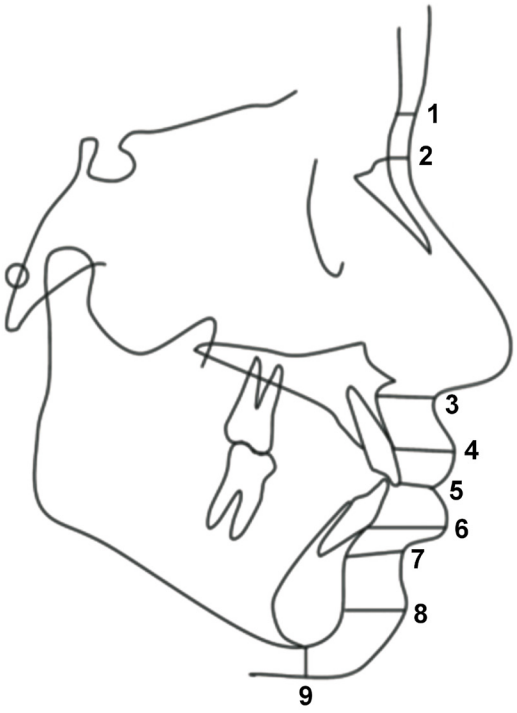

Fig. 2. Schematic view of facial landmarks

1 – G–G'-1; 2 – N–N'; 3 – A–Sn; 4 – Pr–Ls; 5 – J–St; 6 – Id–Li; 7 – B–B'; 8 – Pog–Pog'; 9 – Me–Me'.

(Audax d.o.o., Ljubljana, Slovenia), was used to assess the soft tissue thickness in each group. The lateral cephalograms were evaluated and categorized by an orthodontist and an oral and maxillofacial radiologist. Next, the variables were measured by 2 trained and calibrated examiners using the Romexis® software, v. 3.8.3 (Planmeca, Helsinki, Finland); 25% of the lateral cephalograms were randomly selected and re-assessed after 2 weeks to evaluate intraobserver agreement. The measurements were calibrated using Romexis Viewer. The cephalostat in each image was measured and compared with its actual value of 45 mm to calibrate the images.

Sample size

The sample size was determined to be 36 individuals per skeletal class, in alignment with the methodologies outlined in a previous study conducted by Perović and Blažej.[4] This calculation was based on the assumption that the mean soft tissue thickness at Pr–Ls was 12.75 ±2.50 mm for class II and 14.96 ±3.24 mm for class III patients, with a 95% confidence interval (*CI*) and 80% study power. By considering 20% possible dropouts and applying the correction coefficient, the sample size increased to 72 for each skeletal class (a total of 288 for all 4 classes).

Quantitative variables

The skeletal class (I, II or III) of participants was determined according to the Steiner ANB angle.[1,11,12] The inclination angle (U1–NA angle) was measured to assign class II patients to division 1 or division 2. The ANB angle was measured by identifying the following points:

– point A: lowest point of the line between the anterior nasal spine and the prosthion (alveolar point);
– nasion (N): point at the intersection of the frontal and nasal bones;
– point B: lowest point of the line that connects Id and Pog (midline of the chin).

An ANB angle between 2 and 4 degrees was indicative of class I, values greater than 4 degrees indicated class II, and values less than 2 degrees indicated class III. The U1–NA angle, which was measured to determine the division of class II patients, is formed at the intersection of the longitudinal axis of maxillary central incisor and the nasion plane. The values ≤22 degrees were indicative of division 2, while those exceeding 22 degrees were indicative of division 1.[1,4,6]

The first group of participants (n = 72) exhibited orthognathic class I occlusion, with ANB angle values ranging from 2 to 4 degrees. The second group (n = 71) was categorized as class II division 1, characterized by an ANB angle >4 degrees, and a U1–NA angle >22 degrees. The third group (n = 72) was designated as class II division 2 with distal jaw deviation, an ANB angle >4 degrees, and a U1–NA angle ≤22 degrees. The fourth group (n = 70) was categorized as class III, characterized by an ANB angle <2 degrees.

Statistical analysis

The χ^2 test was used to analyze the correlations between the qualitative variables. The normality of quantitative data was analyzed using the Shapiro–Wilk test. The t-test and analysis of variance (ANOVA) were applied to compare the mean values in each group. Pairwise comparisons were carried out using Tukey's test. The Pearson's correlation test was used to assess interobserver and intraobserver reliability. All statistical analyses were carried out using the SPSS for Windows software, v. 16.0 (SPSS Inc., Chicago, USA). Statistically significant values were defined as $p < 0.05$.

Results

Participants

A total of 285 lateral cephalograms were evaluated, including 144 from females and 141 from males. The sample was comprised of 72 individuals categorized as class I (25.3%; 36 males and 36 females), 71 participants categorized as class II division 1 (24.9%; 35 males, 36 females), 72 patients designated as class II division 2 (25.3%; 36 males, 36 females), and 70 individuals categorized as class III (24.6%; 34 males, 36 females).

Descriptive data

The mean age of the study participants was 25.2 ±2.1 years for males and 24.5 ±3.1 years for females, with a range of 18–40 years. One-way ANOVA revealed no statistically significant difference in the mean age between various skeletal classes ($p = 0.158$).

Outcome data

The mean values in the 4 skeletal classes were compared, irrespective of sex (Fig. 3). Additionally, the analysis of the mean values of the soft tissue thickness was conducted in males and females among different skeletal classes (Fig. 4,5).

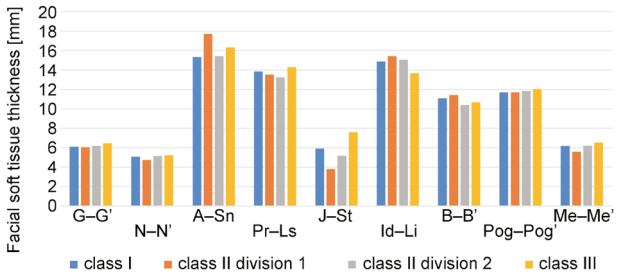

Fig. 3. Comparison of the mean soft tissue thickness at different points across 4 skeletal classes

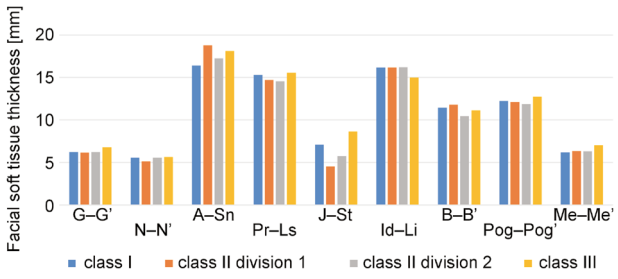

Fig. 4. Comparison of the mean soft tissue thickness at different points in males across 4 skeletal classes

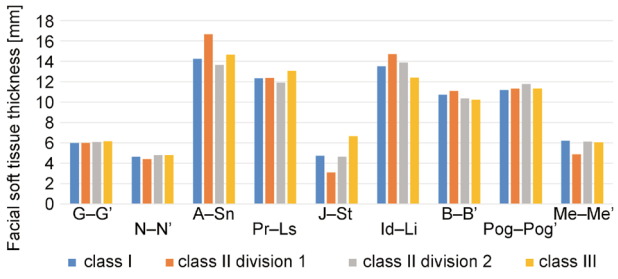

Fig. 5. Comparison of the mean soft tissue thickness at different points in females across 4 skeletal classes

Main results

The results demonstrated an increase in the soft tissue thickness at the subnasale in the class III and class II division 1 groups. Statistically significant differences in the thickness at the stomion were observed among various skeletal classes ($p < 0.05$). Increased thickness at this point was noted in the class I and class III groups, while a decrease in thickness was observed in the class II group. The difference in the thickness of the labrale inferius was more pronounced among the female subjects, as evidenced by the presence of a thinner lower lip across all classes. A significant difference in the soft tissue thickness at the labiomentale was observed between males categorized as class II division 1 and those designated as class II division 2. This finding suggests an increase in the soft tissue thickness at this particular site in the class II division 1 group. The difference in thickness at the pogonion was not significant between males and females in any group except class III. Nonetheless, the thickness at the menton was lower in class II division 1 females. A general observation reveals that the mean thickness of soft tissue was greater in males than in females ($p < 0.05$).

The lowest and highest values for males and females at different anatomical points were as follows: glabella in males (3.6–10 mm) and females (3.5–8.7 mm); nasion in males (2.6–9.8 mm) and females (2.1–8.6 mm); subnasale in males (8.2–29.3 mm) and females (9.8–21.0 mm); labrale superius in males (8.9–21.7 mm) and females (7.4–19.0 mm); stomion in males (1.8–15.9 mm) and females (0.9–15.6 mm); labrale inferius in males (10.7–21.0 mm) and females (9.5–18.1 mm); labiomentale in males (7.8–17.7 mm) and females (7.9–15.5 mm); pogonion in males (4–19.9 mm) and females (5.8–16.4 mm); and menton in males (2.6–13.0 mm) and females (1.5–10.3 mm). Table 1 presents a comparison of the soft tissue thickness between males and females, irrespective of their skeletal class.

Points at the midline exhibited significant differences across all groups and included subnasale, labrale superius, stomion, and labrale inferius ($p < 0.05$). As shown in Table 2, in the skeletal class III individuals, the difference between males and females was more pronounced, and became statistically significant at all points ($p < 0.05$).

The observed variation among the class I individuals did not reach statistical significance at the glabella, labiomentale, pogonion, and menton. In the class II division 1 individuals, the difference between males and females was significant at the subnasale, labrale superius, stomion, labrale inferius, and menton ($p < 0.05$). In class II division 2, the difference was significant only at the subnasale, labrale superius, stomion, and labrale inferius ($p < 0.05$).

Table 3 presents the intra- and interobserver reliability. The intraobserver reliability was assessed using Pearson's correlation coefficients, which demonstrated very strong reproducibility (0.853–0.948). Similarly, the correlation coefficients for the interobserver reliability assessment displayed strong to very strong reproducibility of all measurements after a two-week interval (0.645–0.857).

Table 1. Comparison of the soft tissue thickness between males and females at 9 facial landmarks

Facial landmark	Facial soft tissue thickness [mm] *M* ±*SD*		*p*-value
	males (*n* = 141)	females (*n* = 144)	
G–G′	6.40 ±1.24	6.06 ±1.01	0.030*
N–N′	5.47 ±1.46	4.67 ±1.26	<0.001*
A–Sn	17.62 ±2.79	14.80 ±2.16	<0.001*
Pr–Ls	15.04 ±2.53	12.43 ±1.84	<0.001*
J–St	6.50 ±2.67	4.78 ±2.07	<0.001*
Id–Li	15.90 ±1.93	13.64 ±1.62	<0.001*
B–B′	11.20 ±1.70	10.6 ±1.43	0.001*
Pog–Pog′	12.24 ±2.65	11.41 ±2.08	0.004*
Me–Me′	6.46 ±1.92	5.82 ±1.57	0.002*

* statistically significant ($p < 0.05$, *t*-test); *M* – mean; *SD* – standard deviation; G–G′-1 – glabella region, defined as the linear distance between point G (most prominent point of the frontal bone) and its corresponding soft tissue analog point; N–N′ – nasion region, defined as the linear distance between point N (at the fusion of frontal and nasal bones); A–Sn – subnasale region, defined as the distance between point A (most concave point of the maxilla) and the subnasale (Sn); Pr–Ls – distance between the prosthion (Pr) and the upper lip surface (labrale superius (Ls)); J–St – distance between point J (most labial point of upper incisors) and the contact point of the upper and lower lips (stomion (St)); Id–Li – distance between the infradentale (Id) (most anterior and superior point on the alveolar ridge between lower central incisors) and the lower lip surface (labrale inferius (Li)); B–B′ – distance between point B (most concave point of the mandibular symphysis) and point B′ (labiomentale or the most concave point of the soft tissue of mandibular symphysis); Pog–Pog′ – distance between the pogonion (Pog) or most prominent point of the chin and its soft tissue analog point; Me–Me′ – distance between the most inferior point of the mandible (menton (Me)) and its soft tissue analog point.

Table 3. Assessment of inter- and intraobserver reliability

Facial landmark	Intraobserver reliability		Interobserver reliability	
	Pearson's correlation coefficient	*p*-value	Pearson's correlation coefficient	*p*-value
G–G′	0.849	<0.001*	0.932	<0.001*
N–N′	0.808	<0.001*	0.904	<0.001*
A–Sn	0.645	<0.001*	0.853	<0.001*
Pr–Ls	0.833	<0.001*	0.935	<0.001*
J–St	0.857	<0.001*	0.948	<0.001*
Id–Li	0.772	<0.001*	0.875	<0.001*
B–B′	0.795	<0.001*	0.894	<0.001*
Pog–Pog′	0.823	<0.001*	0.918	<0.001*
Me–Me′	0.809	<0.001*	0.856	<0.001*

* statistically significant ($p < 0.05$).

Discussion

Key results

The present study assessed the soft tissue thickness of an Iranian adult population with different malocclusions according to their lateral cephalograms. The results demonstrated that, in males, the soft tissue thickness at the subnasale in the class III and class II division 1 individuals was significantly greater compared to the class I individuals. Additionally, in males, the soft tissue thickness at the stomion was significantly lower in the class II division 1 individuals due to the proclination of upper

Table 2. Comparison of the soft tissue thickness between males and females in different skeletal classes at 9 facial landmarks (*N* = 285)

Facial landmark	Facial soft tissue thickness [mm] *M* ±*SD*											
	class I			class II division 1			class II division 2			class III		
	males (*n* = 36)	females (*n* = 36)	*p*-value	males (*n* = 35)	females (*n* = 36)	*p*-value	males (*n* = 36)	females (*n* = 36)	*p*-value	males (*n* = 34)	females (*n* = 36)	*p*-value
G–G′	6.2 ±1.2	5.9 ±0.9	0.230	6.2 ±1.1	5.9 ±0.9	0.210	6.2 ±1.3	6.1 ±1.1	0.726	6.8 ±1.3	6.2 ±1.2	0.046*
N–N′	5.6 ±1.4	4.6 ±1.0	0.008*	5.1 ±1.5	4.4 ±1.0	0.023	5.5 ±1.4	4.8 ±1.5	0.044*	5.7 ±1.6	4.8 ±1.1	0.007*
A–Sn	16.4 ±2.5	14.2 ±1.9	<0.001*	18.8 ±3.3	16.6 ±1.2	<0.001*	17.2 ±0.3	13.7 ±1.5	<0.001*	18.1 ±2.6	14.7 ±2.1	<0.001*
Pr–Ls	15.3 ±2.2	12.4 ±1.4	<0.001*	14.7 ±2.5	12.4 ±1.6	<0.001*	14.6 ±2.7	11.9 ±1.9	<0.001*	15.6 ±2.8	13.1 ±2.3	<0.001*
J–St	7.1 ±1.6	4.7 ±1.2	<0.001*	4.5 ±2.2	3.1 ±0.9	<0.001*	5.8 ±1.9	4.6 ±1.5	0.004*	8.7 ±2.9	6.6 ±2.6	0.002*
Id–Li	16.2 ±2.0	13.5 ±1.2	<0.001*	16.2 ±1.8	14.7 ±1.2	<0.001*	16.2 ±1.7	13.9 ±1.6	<0.001*	15.0 ±2.1	12.4 ±1.9	<0.001*
B–B′	11.4 ±1.8	10.7 ±1.4	0.069	11.8 ±1.7	11.1 ±1.5	0.068	10.4 ±1.2	10.4 ±1.3	1.000	11.1 ±1.8	10.2 ±1.4	0.021*
Pog–Pog′	12.2 ±2.6	11.2 ±1.9	0.067	12.1 ±2.7	11.3 ±2.2	0.173	11.8 ±2.2	11.8 ±1.8	1.000	12.8 ±3.6	11.3 ±2.3	0.039*
Me–Me′	6.2 ±1.9	6.2 ±1.5	1.000	6.4 ±1.8	4.9 ±1.6	<0.001*	6.3 ±1.9	6.12 ±1.5	0.657	7.1 ±2.1	6.1 ±1.3	0.018*

* statistically significant ($p < 0.05$, ANOVA).

incisors. An increase in thickness at this point was noted in the class I and class III subjects. The increase in the class III group was so high that the difference between class III and class I became statistically significant. On the other hand, lower lip thickness in class III exhibited a significant difference compared to the class I and class II division 2 groups, and class II division 1. A significant difference in labiomentale was also identified between the class II division 1 and 2 individuals. Similar results were noted in the female subjects. At the subnasale, a difference in thickness was identified between class II division 1 and class I, as well as between the class III and class II division 2 groups. An increase in thickness at this point was noted in class III and class II division 1, while a reduction in thickness was identified in the class II division 2 and class I groups. As observed at the stomion, the differences among the various skeletal classes in females were similar to those reported for males. At the labrale inferius, significant differences were noted between the majority of the groups. The thickness was higher in class II and lower in the class III individuals. At the menton, the class II division 1 group showed significantly lower thickness than the other groups.

The present results revealed a significantly higher soft tissue thickness in males at all points, irrespective of their skeletal classes. The comparison of the soft tissue thickness between males and females in each skeletal class demonstrated that the mean soft tissue thickness in males was higher than in females at all points.

Interpretation

Kamak and Celikoglu found significant differences in the facial soft tissue thickness among the 3 skeletal classes at the labrale superius, stomion and labiomentale in a Turkish population.[13] Utsuno et al. revealed significant differences in the soft tissue thickness among different skeletal classes at the subnasale, labrale superius, stomion, labiomentale, and pogonion in Japanese females.[14] Jeelani et al. conducted a study on a Pakistani population, which identified substantial variations in the soft tissue thickness among different skeletal classes at the glabella, labrale superius and stomion in males and labrale superius, labrale inferius, labiomentale, and pogonion in females.[9] Sarilita et al. indicated significant differences in the soft tissue thickness between the class II and class III groups at the lower lip in males, and at the subnasale, upper and lower lips, stomion, and labiomentale in females.[5] They also confirmed a general pattern of an increased lower lip thickness in class II compared with class III individuals, as well as a thicker upper lip in class III compared to class II subjects.[5]

The observed differences between the present findings and the results of previous studies can be attributed to racial differences among the study populations. Bacon et al. in their review confirmed the presence of a difference in soft tissue profile among different ethnic and racial groups.[15] Additionally, the difference in the soft tissue thickness among various classes was not significant at points where soft tissue was close to the underlying bone, such as the nasion and the pogonion. Similar results were reported by Kurkcuoglu et al.[16] and Jeelani et al.[9] The thickness of the facial soft tissue decreased at the pogonion and labiomentale with mandibular prognathism.[17] This observation was replicated at the labiomentale in the present study.

A comparison of 4 skeletal classes revealed that the class III and class II division 1 groups exhibited an increased soft tissue thickness at the subnasale. This phenomenon may be attributed to hypoplasia of the base of the maxilla in such individuals. In the case of patients exhibiting a routine developmental jaw disproportion, the overlying soft tissue envelope is typically normal, albeit distorted by the underlying skeletal disharmony. On the other hand, normal variations in skin thickness and quality, sweat gland density, fat distribution, and pigmentation are to be expected. An example of soft tissue distortion, as opposed to deformity, is seen in maxillomandibular deficiency (i.e., short face growth pattern). The presence of chubby cheeks and excess fat in the neck is a common observation. The restoration of normal facial contours and curvatures is achieved through maxillomandibular (skeletal) advancement and vertical lengthening.[18] Moreover, class III showed an increased soft tissue thickness at the labrale superius and stomion, accompanied by a decreased soft tissue thickness at the labrale inferius and labiomentale. It has been observed that the pattern of increased upper lip thickness and decreased lower lip thickness can contribute to the camouflage of the underlying skeletal discrepancy. Similarly, decreased upper lip thickness and increased lower lip thickness in class II patients serve to camouflage the skeletal class II relationship.[9]

The class III group demonstrated an increased soft tissue thickness at the glabella. However, these results did not attain statistical significance. This difference was particularly pronounced in class III males when compared to females, a finding that may be attributable to the larger frontal sinus dimensions observed in this skeletal group and in males.[19–23] Yassaei et al. noted a significant correlation between the frontal sinus dimensions and mandibular body length, suggesting that the dimensions of the frontal sinus can serve as an index to assess the remaining growth of the mandible.[19] Yet, further studies are necessary to confirm this statement. The difference in the soft tissue thickness at the glabella may also be due to frontal bossing, a consequence of mandibular prognathism. These conditions are prevalent among individuals with elevated growth hormone secretion levels.[24]

Changes in the facial soft tissue thickness can be related to the inclination angle of upper incisors. Accordingly, in the class II division 1 cases, the lower lip has a proclined and downward position, affecting the soft tissue thick-

ness at the labiomentale and labrale inferius. Moreover, the significant difference in the soft tissue thickness at the stomion between the class II division 1 and 2 groups is associated with the inclination angle.

Jeelani et al. conducted a study on Pakistani adults, in which they reported significantly higher soft tissue thickness at the glabella, nasion, subnasale, labrale superius, and labrale inferius in males.[9] Sarilita et al. evaluated an Indonesian population and reported a significantly higher soft tissue thickness in males at the nasion, rhinion, subnasale, upper and lower lips, and stomion.[5] Uysal et al. found significant differences in the soft tissue thickness at the labrale superius, labrale inferius, pogonion, and menton between male and female Turkish adults.[11] Kamak and Celikoglu observed a significantly higher soft tissue thickness at all points except for the glabella, labiomentale and pogonion in Turkish males.[13] Furthermore, Utsuno et al. assessed Japanese adults and found sex-related differences in the facial soft tissue thickness at all points between the skeletal class I and III groups.[14]

The present study assessed the soft tissue thickness between males and females separately in each skeletal class. The results demonstrated that the mean soft tissue thickness in males was higher than in females at all points. The differences were particularly pronounced in class III at all measured points. Nonetheless, the differences were not significant in some groups at specific points when the distance from the underlying bone was minimal.

Few studies utilized cone beam computed tomography (CBCT) to evaluate soft tissue dimensions in malocclusion patients. Jazmati et al. evaluated the cone-beam computed tomography (CBCT) scans of 96 patients in 3 skeletal sagittal classes.[25] In male patients, the values of U1-stom, nasal width and mouth width were significantly greater in class I as compared to class II patients. Also, class I patients had a lower lip thickness than class III patients. In female patients, the upper lip height and the labiomental fold thickness were significantly greater in class II in comparison with class I patients. Conversely, class I patients had a greater lower lip height than class III patients. Class III patients had greater Ls-Pr, U1-stom and face width at Cheilion as compared to class II patients. Class II patients indicated lower values of the lip thickness, the lower lip height and the upper lip height as compared to class III patients. Males had a greater soft tissue thickness than females. Nonetheless, the differences between males and females in the variables assessed in each skeletal class did not reach statistical significance.[25]

It is important to acknowledge that congenital deformities can influence the facial soft tissue thickness, as evidenced by cleft palates. Paradowska-Stolarz et al. assessed the relationship between clefts, determining that cleft palate may influence soft tissue formation and can be limited to the uvula.[26] There are exceptions where the soft tissue envelope is directly affected due to a malformation (e.g., Treacher Collins syndrome, hemifacial microsomia, cleft lip, hemifacial hypertrophy), previous trauma (burns, lacerations), or scarring after infection. In cases where a jaw deformity is associated with a syndrome, clefting, or a soft tissue traumatic deformity, the impact on facial aesthetics and head and neck functions is exacerbated, and the soft tissue envelope necessitates particular consideration.[18] The present study encompassed healthy individuals who did not suffer from any congenital disease.

Generalizability

The significance of soft tissue analysis and determination of skeletal class prior to facial reconstructions is further highlighted in the presence of a significant difference in the soft tissue thickness at different anatomical points among the 4 skeletal classes. Also, it is important to take into account the differences in the soft tissue thickness between males and females, as well as among different skeletal classes, in the context of orthodontic treatment planning.

Limitations

The present study was subject to certain limitations. It has been documented that the body mass index (BMI) may have an impact on the soft tissue thickness.[7] However, the retrospective design of the present study precluded the control of this confounder. Additionally, the study was conducted in 1 city; thus, the results may not be generalizable to the entire population of Iran. Future studies should compare the soft tissue thickness of different ethnic groups within the Iranian population.

Conclusions

A thicker facial soft tissue was observed in males compared to females across all skeletal classes, and this difference was more pronounced in the class III individuals. A statistically significant variation in the soft tissue thickness was observed among different skeletal classes, particularly at regions that were farther from the underlying bone. The class III individuals exhibited increased thickness in the upper lip soft tissue, accompanied by a decrease in the lower lip soft tissue thickness. In the class II individuals, a decrease in upper lip thickness and an increase in lower lip thickness were observed. This pattern suggests that the underlying skeletal discrepancy is being concealed.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Qazvin University of Medical Sciences, Iran (IR.QUMS.REC.1401.197).

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Milad Ali Asgharlou https://orcid.org/0000-0003-1301-3719
Mahsa Esfahani https://orcid.org/0000-0001-6896-0487
Maryam Tofangchiha https://orcid.org/0000-0002-5515-2189
Amir Javadi https://orcid.org/0000-0002-3365-9042
Nima Sheikhdavoodi https://orcid.org/0000-0001-9715-5153
Zahra Yousefi https://orcid.org/0000-0002-3690-8251
Rodolfo Reda https://orcid.org/0000-0003-1532-6524
Luca Testarelli https://orcid.org/0000-0003-3904-3000

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Original paper

Analyzing and exploring Graph Attention Networks and protein-based language models for predicting *Porhyromonas gingivalis* resistant efflux protein sequences

Pradeep Kumar Yadalam[1,A], Prabhu Manickam Natarajan[2,B], Naresh Shetty[3,C], Maria Maddalena Marrapodi[4,D], Hande Uzunçıbuk[5,D], Diana Russo[6,E], Marco Cicciù[7,E,F], Giuseppe Minervini[8,9,D,E]

[1] Department of Periodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS Deemed University), Chennai, India
[2] Department of Clinical Sciences, Center of Medical and Bio-Allied Health Sciences and Research, Ajman University, UAE
[3] Department of Clinical Sciences, College of Dentistry, Ajman University, UAE
[4] Department of Woman, Child and General and Specialist Surgery, University of Campania Luigi Vanvitelli, Naples, Italy
[5] Department of Orthodontics, Faculty of Dentistry, Trakya University, Edirne, Turkey
[6] Oral Surgery Unit, Multidisciplinary Department of Medical-Surgical and Dental Specialties, University of Campania Luigi Vanvitelli, Naples, Italy
[7] Department of Biomedical and Surgical and Biomedical Sciences, Catania University, Italy
[8] Department of Orthodontics, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS Deemed University), Chennai, India
[9] Multidisciplinary Department of Medical-Surgical and Dental Specialties, University of Campania Luigi Vanvitelli, Naples, Italy

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):265–273

Address for correspondence
Giuseppe Minervini
E-mail: giuseppe.minervini@unicampania.it

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on February 21, 2024
Reviewed on February 27, 2024
Accepted on March 15, 2024

Published online on April 30, 2025

Cite as
Yadalam PK, Natarajan PM, Shetty N, etc. Analyzing and exploring Graph Attention Networks and protein-based language models for predicting *Porhyromonas gingivalis* resistant efflux protein sequences. *Dent Med Probl.* 2025;62(2):265–273. doi:10.17219/dmp/186143

DOI
10.17219/dmp/186143

Abstract

Background. Antimicrobial resistance (AMR) must be predicted to combat antibiotic-resistant illnesses. Based on high-priority AMR genomes, it is possible to track resistance and focus treatment to stop global outbreaks. Large language models (LLMs) are essential for identifying *Porhyromonas gingivalis* multi-resistant efflux genes to prevent resistance. Antibiotic resistance is a serious problem; however, by studying specific bacterial genomes, we can predict how resistance develops and find better kinds of treatment.

Objectives. This paper explores using advanced models to predict the sequences of proteins that make *P. gingivalis* resistant to treatment. Understanding this approach could help prevent AMR more effectively.

Material and methods. This research utilized multi-drug-resistant efflux protein sequences from *P. gingivalis*, identified through UniProt ID A0A0K2J2N6_PORGN, and formatted as FASTA sequences for analysis. These sequences underwent rigorous detection and quality assurance processes to ensure their suitability for computational analysis. The study employed the DeepBIO framework, which integrates LLMs with deep attention networks to process FASTA sequences.

Results. The analysis revealed that the Long Short-Term Memory (LSTM)-attention, ProtBERT and BERTGAT models achieved sensitivity scores of 0.9 across the board, with accuracy rates of 89.5%, 88.5% and 90.5%, respectively. These results highlight the effectiveness of the models in identifying *P. gingivalis* strains resistant to multiple drugs. Furthermore, the study assessed the specificity of the LSTM-attention, ProtBERT and BERTGAT models, which achieved scores of 0.89, 0.87 and 0.90, respectively. Specificity, or the genuine negative rate, measures the ability of a model to accurately identify non-resistant cases, which is crucial for minimizing false positives in AMR detection.

Conclusions. When utilized clinically, this LLM approach will help prevent AMR, which is a global problem. Understanding this approach may enable researchers to develop more effective treatment strategies that target specific resistant genes, reducing the likelihood of resistance development. Ultimately, this approach could play a pivotal role in preventing AMR on a global scale.

Keywords: periodontitis, antimicrobial resistance, large language models, *Porphyromonas gingivalis*, efflux protein

Highlights

- By examining efflux protein sequences, advanced artificial intelligence (AI) models, such as LSTM-attention, ProtBERT and BERTGAT, can accurately (up to 90.5%) and sensitively (~0.90) predict antimicrobial resistance (AMR) in *Porphyromonas gingivalis*.
- With the highest accuracy (90.5%) and specificity (0.90), BERTGAT performed better than other models. This indicates that adding graph-based attention mechanisms enhances AMR prediction by more accurately capturing biological relationships.
- The SHAP, UMAP, ROC, PR, and UpSet plots confirmed model interpretability and robustness, indicating their possible clinical use in detecting resistant strains and directing precise antibiotic tactics.
- The study emphasizes the significance of targeting efflux proteins for novel drug design to combat multidrug-resistant *P. gingivalis*, a keystone pathogen in periodontitis.
- Notwithstanding encouraging findings, the small dataset size and the absence of external validation are drawbacks that call for additional research with bigger and more varied datasets.

Introduction

Antimicrobial resistance (AMR)[1,2] is the ability of microorganisms to resist the effects of antimicrobial drugs, such as antibiotics, antivirals and antiparasitics.[3–5] Combating antibiotic-resistant diseases requires predicting AMR. High-priority AMR genomes can lead surveillance to track resistance and focus treatment in order to prevent global outbreaks.[6–8]

Leveraging insights from large language models (LLMs), like ProtBERT or BERTGAT, can be employed to explore the intricate mechanisms governing the interplay between protein sequences, their structural configurations and resultant functions.[9,10] The essence of this paradigm lies in understanding how the linear arrangement of amino acids, akin to the syntax of a sentence, dictates the three-dimensional (3D) structure of a protein, which, in turn, governs its biological functions. By adopting computational language models, traditionally used in natural language processing (NLP), we gain a valuable tool to dissect and decipher the functions of proteins.[11–13] This approach allows researchers to unveil the nuanced relationships between amino acid sequences, the structural motifs they form and the functional roles they play in biological processes. Treating protein sequences as linguistic entities provides a powerful framework for unraveling the language of life encoded in these fundamental biological molecules.[14]

The attention-based Long Short-Term Memory (LSTM-attention) network is a method that analyzes big datasets and looks for patterns that point to AMR, using state-of-the-art algorithms.[15–22] Co-AMPpred is one instance of a machine learning method for AMR prediction.[23,24] This tool distinguishes between antimicrobial peptides (AMPs) and non-AMPs by combining physicochemical characteristics and composition-based sequences through machine learning techniques.

An important global health concern is periodontitis, an immune-inflammatory infectious disease, mostly caused by *Porphyromonas gingivalis*.[25,26] The bacterium exhibits a variety of omics and phylogeny information, making it a significant factor in severe periodontitis. Treatment for *P. gingivalis* is becoming more difficult due to its growing resistance to antibiotics, which highlights the need for a deeper comprehension of its resistance mechanisms. In particular, the resistance-nodulation-division (RND) family of efflux pumps is a major contributor to the AMR of *P. gingivalis*. These pumps, including proteins such as AcrA, AcrB and TolC,[27–30] block the entry of antimicrobial drugs into the bacterial cell, contributing to multi-drug resistance (MDR).

Porphyromonas gingivalis-produced gingipains and virulence factors[31,32] add to the complexity of the situation. Due to gingipains, *P. gingivalis* can elude the host immune system, which contributes to AMR. The integrated protein–protein interaction network (PPIN), which includes virulence regulators and efflux pump proteins, was subjected to topological and functional analysis; this analysis identified genes crucial for understanding the relationships across cellular systems in *P. gingivalis*.[31] The bifunctional NAD(P)H-hydrate repair enzyme A0A212GBI3_PORGN is one of the most prevalent resistant efflux proteins.[33–37] It is essential for the bifunctional enzyme that it catalyzes the dehydration of the S-form of NAD(P)HX[38] at the expense of ADP, which is converted to AMP, as well as the epimerization of the S- and R-forms of NAD(P)HX.

Identifying *P. gingivalis* multi-resistant efflux genes with the use of LLMs is crucial for preventing resistance. The present study aimed to analyze and explore Graph Attention Networks (GATs) and protein-based language models for predicting *P. gingivalis* resistant efflux protein sequences.

Methods

Using UniProt,[39] the following sequences of multi-drug resistant proteins of *P. gingivalis* were downloaded: A0A0K2J2N6_PORGN; A0A212GBI3_PORGN;

A0A2D2N4E3_PORGN; A0A0E2LNT1_PORGN; A0A829KLL9_PORGN; U2K1P7_PORGN; Q7MXT9_PORGI; A0A1R4DUJ6_PORGN; and A0A212FQN2_PORGN. The identified FASTA sequences underwent a thorough quality check to ensure that there were no biases during their entry. Additionally, the sequences were formatted according to the prescribed format based on the DeepBIO tool for LLMs and deep attention networks.[40]

DeepBIO

Academics can construct a deep learning architecture to address any biological problem with the help of DeepBIO, a one-stop web service. In addition to visualizing biological sequencing data, DeepBIO compares and enhances deep learning models. It offers base functional annotation tasks, with in-depth interpretations and graphical visualizations, and conservation motif analysis to confirm site dependability, and well-trained deep learning architectures for more than 20 tasks. The sequence-based datasets were divided into the training and test sets using DeepBio. We randomly divided each dataset into 1,000 training and 200 testing sets to optimize hyperparameters and analyze performance.

BERTGAT

BERTGAT[41] is a neural network model that combines the pre-trained language model Bidirectional Encoder Representations from Transformers (BERT) with GAT.[16,42] BERT extracts text features,[41] while GAT learns the sentence–word relationships.[26,43,44] Transformer-based language models are preferred over recurrent neural networks (RNNs). Pre-trained BERT representations are fine-tuned to generate state-of-the-art models for wide-ranging text-to-structured query language (SQL) workloads with one extra output layer.

ProtBERT

The provided search results do not contain specific information about the full code architecture of ProtBERT and its detailed steps. However, based on the available information, it was possible to provide a general outline of the architecture and the steps involved in using ProtBERT for protein sequence prediction.[41]

ProtBERT architecture and steps for protein sequence prediction

Pre-training

ProtBERT is pre-trained on a large dataset of protein sequences, representing the entire known protein space, using a masked language modeling task combined with a novel Gene Ontology (GO) annotation prediction task. The architecture of ProtBERT consists of local and global representations, allowing the end-to-end processing of protein sequences and GO annotations.

Fine-tuning

After pre-training, the ProtBERT model is fine-tuned on specific protein-related tasks, such as protein sequence classification or function prediction. Fine-tuning involves initializing the model from the pre-trained state, freezing some layers, training additional, fully connected layers, and then unfreezing all layers for further training.

Model evaluation

The fine-tuned ProtBERT model is evaluated on diverse benchmarks covering various protein properties to assess its performance. The ProtBERT model is built on Keras/TensorFlow and is available through the Hugging Face model hub. The code for using ProtBERT involves loading the pre-trained model, fine-tuning it on specific protein-related tasks, and utilizing it for protein sequence prediction and analysis.

LSTM-attention model

LSTM[15,17] and attention mechanisms are combined in LSTM-attention, a deep learning architecture, to enhance sequence prediction task performance. The following steps are needed to put the LSTM-attention model into practice:

1. Data Preparation: The first stage is to prepare the input data for the model. This could entail activities like feature extraction, encoding and tokenization.
2. Model Architecture: An LSTM layer and an attention layer form the LSTM-attention model. After processing the input sequence, the LSTM layer creates a series of hidden states. The more pertinent states are given more weight when the attention layer computes a weighted sum of the hidden states.
3. Training: The model is trained using the appropriate loss function and optimization technique with the prepared data. The parameters of the model are adjusted during training to minimize the loss function.
4. Evaluation: After training, the performance of the model is assessed on an independent test set. This entails calculating metrics like the F1 score, recall, accuracy, and precision.
5. Prediction: The model can forecast new sequences after evaluation. The trained model receives the input sequence and the learned weights generate the output.
6. Fine-tuning: The model can be further adjusted on particular tasks or datasets to boost performance. This involves changing the hyperparameters or architecture of the model to fit a given task better (Table 1).

Table 1. Parameters of the Protein Language Model (PLM)

Cuda:	TRUE	TRUE2	TRUE3
Seed:	43	43	43
num_workers:	4	4	4
num_class:	2	2	2
Kmer:	3	3	3
heatmap_seq:			
save_figure_type:	png	png	png
Mode:	train-test	train-test	train-test
Type:	prot	prot	prot
model:	BertGAT	LSTMAttention	prot_bert
datatype:	userprovide	userprovide	userprovide
interval_log:	10	10	10
interval_valid:	1	1	1
interval_test:	1	1	1
Epoch:	50	50	50
Optimizer:	Adam	Adam	Adam
loss_func:	CE	CE	CE
batch_size:	4	8	32
LR:	1.00E-05	0.0001	0.0001
Reg:	0.0025	0.0025	0.0025
Gamma:	2	2	2
Alpha:	0.25	0.25	0.25
max_len:	35	207	52
dim_embedding:	32	32	32
minimode:	modelCompare	modelCompare	modelCompare
if_use_FL:	0	0	0
if_data_aug:	0	0	0
if_data_enh:	0	0	0
CDHit:	['1']	['1']	['1']

Results

LSTM-attention, ProtBERT and BERTGAT were used to find the hidden features and weights in the FASTA protein sequences; then, backpropagation algorithms with ADAM optimizer and 50 iterations fine-tuned the model.

LSTM-attention, ProtBERT and BERTGAT had sensitivity of 0.90, 0.90 and 0.91, respectively (TP / (TP + FN); TP – true positive, FN – false negative). Specificity, or the true negative rate, is the proportion of actual negatives correctly predicted as negatives. The specificity of LSTM-attention, ProtBERT and BERTGAT was 0.89, 0.87 and 0.90, respectively (TN / (TN + FP); TN – true negative, FP – false positive).

ROC curve

The receiver-operating characteristic (ROC) curve shows the trade-off between the true positive rate (sensitivity) and false positive rate (1-specificity) of the model over the categorization thresholds. Regarding LSTM-attention, ProtBERT and BERTGAT, high true positive rates are shown by the ROC curve in the upper left corner of the plot.

PR curve

The trade-off between recall and precision for binary classifiers with different probability thresholds is depicted by the precision–recall (PR) curve. While precision is the fraction of positive predictions, recall is the percentage of accurately expected positives. This model's performance with uneven classes is made public. The area under the PR curve (AUC-PR) is a widely used metric to summarize the classifier performance. Higher AUC-PR values for LSTM-attention, ProtBERT and BERTGAT denote improved model performance.

An epoch plot is a graph showing the accuracy and loss of a machine learning model over training. It is an effective diagnostic tool for overfitting and other model issues. The number of epochs or iterations the model has been trained on is shown by the X-axis in an epoch plot. The accuracy or loss of the model is plotted on the Y-axis. The loss indicates how effectively the model predicts the proper output for a given input. Accuracy gauges whether the predictions of the model are accurate.

UpSet plot

The frequency of common items between groups can be ascertained by comparing the intersection diameters. While smaller crossings imply less overlap, larger intersections show more overlap between groups. In a vertical UpSet plot, rows represent intersections and matrix columns represent sets. Each row has filled intersection cells that show how the rows are related to each other.

Uniform Manifold Approximation and Projection (UMAP) creates a weighted graph from high-dimensional data to show clustering patterns, with the edge strength reflecting how 'close' the points are. Projecting this graph lowers its dimension. This data shows algorithm clustering. UMAP is a non-linear dimension reduction method for embedding high-dimensional data in low-dimensional space. It assumes that high-dimensional data points should be close to low-dimensional space.

SHAP values

The predictive value of each feature is quantified in a machine learning model. All possible feature combinations are considered, along with the relative contributions of each feature to the prediction when coupled with a subset of features, to compute the value. When a feature enhances the prediction, the Shapley Additive Explanations (SHAP) red value is positive. A feature with a negative SHAP blue value is less predictive.

Discussion

Antimicrobial drug-resistant periodontal bacteria[45–47] are characterized by efflux pumps – proteins that remove antimicrobial medications from the cell, thus preventing the drugs from killing the bacteria. Bacteria can also adapt their outer membrane to block antimicrobial medications or change the target site of the drug to lessen its efficacy.[36,48] These pathways and others cause antibiotic resistance in periodontitis patients. Whole-genome sequencing can detect AMR genes[34,35,49,50] and mutations, assessing the resistance potential. Large genomic, phenotypic and clinical datasets can be used to train machine learning algorithms to predict resistance and discover the key AMR genes. Prolonged illness, more expensive second-line therapies and missed productivity can strain healthcare systems and national economies. Predicting AMR in globally prevailing periodontal infections, especially for the keystone pathogens like *P. gingivalis*, is important for preventing resistance from spreading across continents. Antimicrobial resistance is a growing concern in the field of periodontitis research. It refers to the ability of microorganisms, such as bacteria, to resist the effects of antimicrobial drugs.[1,51]

Large language models have revolutionized various fields, including protein sequence prediction. In this study, models such as LSTM-attention, ProtBERT and BERTGAT demonstrated high predictive performance, with accuracy rates reaching up to 90.5% (Table 2, Fig. 1–5). Large language models have also shown strong results in broader protein-related tasks, such as structure prediction and protein design, in previous studies.

The observed performance differences between LSTM-attention, ProtBERT and BERTGAT, with accuracy rates of 89.5%, 88.5% and 90.5%, respectively, deepen the

Table 2. Accuracy of the LSTM-attention, ProtBERT and BERTGAT models

Model name	Accuracy	Sensitivity	Specificity	AUC
LSTM-attention	0.895	0.90	0.89	0.948
ProtBERT	0.885	0.90	0.87	0.941
BERTGAT	0.905	0.91	0.90	0.951

AUC – area under the curve (referring the receiver-operating characteristic (ROC) curve unless otherwise specified). Accuracy is defined as the overall proportion of correctly classified cases (true positives and true negatives) among all predictions.

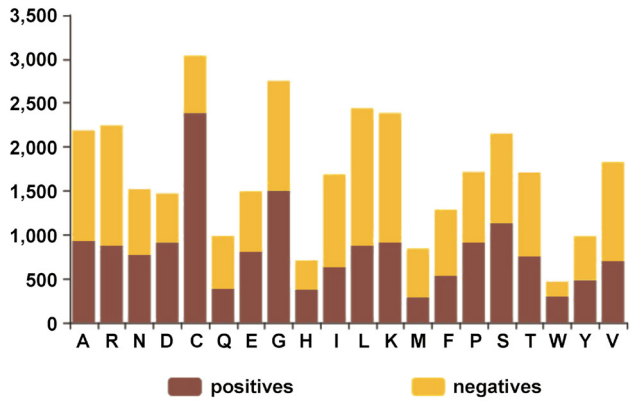

Fig. 1. Distribution of positive (antimicrobial drug-resistant) and negative (non-resistant) sequences in the training and test datasets

The letters on the X-axis represent the subsets or identifiers of the input sequences used during model training and evaluation.

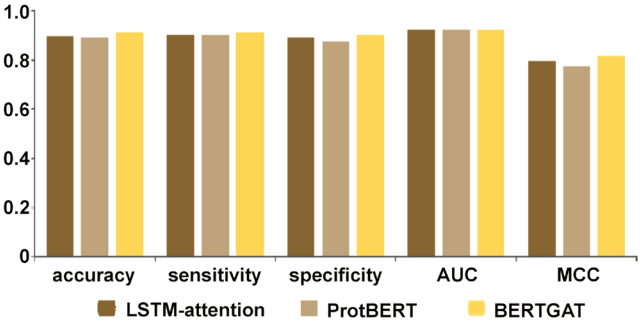

Fig. 2. Accuracy of the models

MMC – Matthews correlation coefficient.

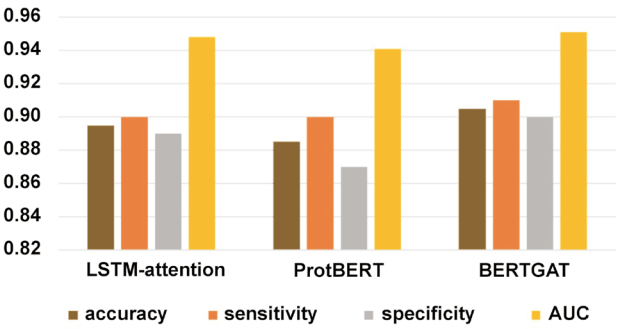

Fig. 3. Bar chart of the accuracy of the models

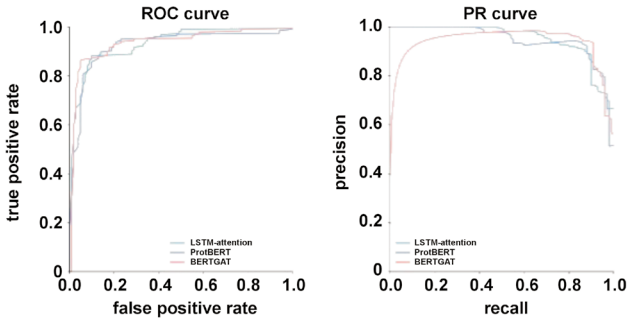

Fig. 4. Receiver-operating characteristic (ROC) and precision–recall (PR) curves of the plot

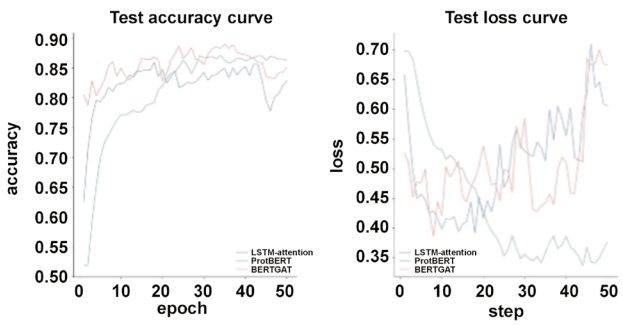

Fig. 5. Epoch plot of all iterations with algorithms

interpretation of the results in the context of model architecture implications.[52,53] LSTM-attention utilizes long short-term memory units and attention mechanisms, while ProtBERT incorporates a transformer-based architecture, specifically designed for protein sequence data, and BERTGAT incorporates graph attention mechanisms. The higher accuracy of BERTGAT suggests that its increased model complexity and ability to capture graph structures in the data have contributed to improved performance. Data representation is another important factor to consider. The comparable accuracy of LSTM-attention and ProtBERT suggests that their respective data representations are effective for a given task. Biological relevance is a critical consideration when evaluating model performance. Protein sequence analysis is inherently tied to biology, and it is important to assess how well the models align with biological knowledge.[50,52,54] While all 3 models demonstrated high accuracy, it is necessary to delve deeper into the interpretation to understand if the superior accuracy of BERTGAT is biologically relevant or if other factors drive it. Overall, the observed performance differences between LSTM-attention, ProtBERT and BERTGAT highlight the impact of model complexity, data representation and biological relevance. Further analysis and interpretation are required to uncover the specific advantages of each architecture and their implications in the context of protein sequence analysis.

Previous state-of-the-art models, like ProteinBERT,[55–57] a universal deep learning model for protein sequences, leveraging the transformer architecture,[58–60] are commonly used in NLP tasks. In addition to language models, various machine learning methods and algorithms are used in protein sequence prediction, such as graph neural networks and deep learning-based algorithms like BERTGAT and LSTM-attention.[15–17] ProtBERT is a transformer-based language model trained on a large corpus of protein sequences to learn representations that capture important structural and functional information.[24] This study compared LLMs vs. GAT-based algorithms[57,61,62] in predicting AMR sequencing, and the performance of the model was shown using the SHAP,[63–65] UMAP and UpSet plot analysis (Fig. 6–8), similar to previous studies for performance.

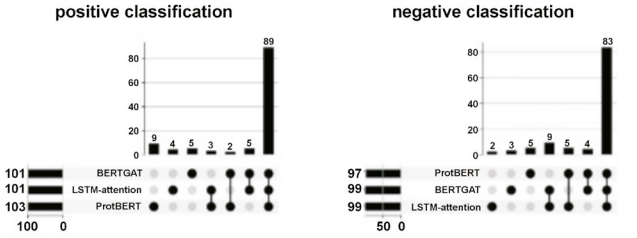

Fig. 6. UpSet intersection diagram of the models with positive and negative classification

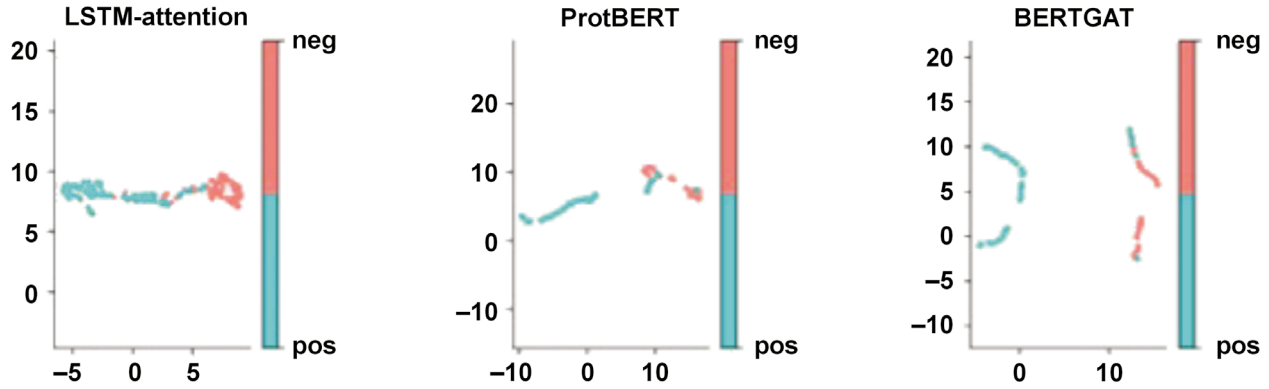

Fig. 7. Uniform Manifold Approximation and Projection (UMAP) plot of the models
neg – negative; pos – positive.

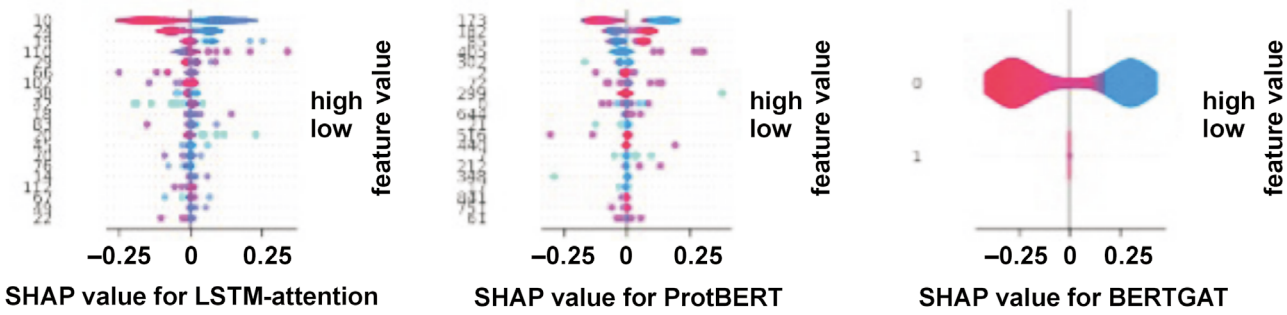

Fig. 8. Shapley Additive Explanations (SHAP) performance of the models

Targeting *P. gingivalis* efflux proteins is important for novel antibiotic drug design. These prediction models could point to resistance mutation sequences and prevent the development of AMR in periodontitis patients.[66,67]

This study compared the performance of LLMs and GAT-based algorithms in predicting AMR sequencing. The model's performance was evaluated using the SHAP, UMAP and UpSet plot analysis, previously employed to assess the performance of similar prediction models. The study also highlighted the significance of targeting *P. gingivalis* efflux proteins to design novel antibiotic drugs. However, it is important to acknowledge that the current study has limitations. One major limitation is the small sample size and the lack of the external validation of the independent datasets used in the study.[52] Future research should address this limitation by including larger sample sizes to ensure the reliability and generalizability of the prediction model. Furthermore, further investigations are needed to validate the model's performance in diverse datasets and to explore its applicability for other oral microbes.

Conclusions

Preventing the spread of antimicrobial resistance (AMR) is a primary global concern, and large language models (LLMs), when applied clinically, may help prevent this phenomenon.

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.

ORCID iDs

Pradeep Kumar Yadalam https://orcid.org/0000-0002-6653-4123
Prabhu Manickam Natarajan https://orcid.org/0000-0002-4780-0465
Naresh Shetty https://orcid.org/0000-0002-4596-5215
Maria Maddalena Marrapodi https://orcid.org/0000-0002-9494-6942
Hande Uzunçıbuk https://orcid.org/0000-0001-9265-1772
Diana Russo https://orcid.org/0000-0002-0915-4642
Marco Cicciù https://orcid.org/0000-0003-2311-9728
Giuseppe Minervini https://orcid.org/0000-0002-8309-1272

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Original paper

Effect of phosphorylated chitosan and carbodiimide on the surface wettability, surface free energy and surface morphology of the eroded dentin

Mirian Saavedra Lopes Ururahy[1,A,D–F], Erick Silva Barbosa[1,B,D,F], Rafaela Manente[2,C,D,F], Ana Paula Ramos[3,B,C,F], Antônio Eduardo Miller Crotti[3,C,E,F], Silmara Aparecida Milori Corona[1,A,E,F]

[1] Department of Restorative Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
[2] Department of Pediatric Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil
[3] Department of Chemistry, Faculty of Philosophy, Sciences and Letters, University of São Paulo, Ribeirão Preto, Brazil

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):275–283

Address for correspondence
Silmara Aparecida Milori Corona
E-mail: silmaracorona@forp.usp.br

Funding sources
The research scholarship funded by the Brazilian National Council for Scientific and Technological Development (CNPq) and the Coordination for the Improvement of Higher Education Personnel (CAPES) (grant No. 144533/2016-2), and a grant from the São Paulo Research Foundation (FAPESP) (grant No. 2017/18150-0).

Conflict of interest
None declared

Acknowledgements
None declared

Received on April 25, 2023
Reviewed on July 18, 2023
Accepted on August 1, 2023

Published online on April 30, 2025

Cite as
Ururahy MSL, Barbosa ES, Manente R, Ramos AP, Crotti AEM, Corona SAM. Effect of phosphorylated chitosan and carbodiimide on the surface wettability, surface free energy and surface morphology of the eroded dentin. *Dent Med Probl.* 2025;62(2):275–283. doi:10.17219/dmp/170245

DOI
10.17219/dmp/170245

Abstract

Background. Biomodifiers can reinforce the collagen matrix, improving the biomechanical and biochemical properties of dentin.

Objectives. The present study aimed to evaluate how 2.5% phosphorylated chitosan (P-Chi) and 0.5 mol/L carbodiimide (EDC) affect the surface wettability, surface free energy and surface morphology of the eroded dentin.

Material and methods. Bovine tooth specimens ($N = 144$) were randomly divided into 6 groups according to the dentin substrate (sound or eroded) and surface treatment (2.5% P-Chi, 0.5 mol/L EDC or no biomodification (control)). Half of the specimens ($n = 72$) were submitted to erosive challenge by immersion in 0.3% citric acid (pH 3.2) for 2 h. For the surface wettability analysis ($n = 12$), the contact angles between the dentin surface and the 3M™ Scotchbond™ Universal adhesive were measured with a goniometer. For the surface free energy analysis ($n = 3$), the contact angles between the dentin surface and 3 organic solvents of distinct polarities (water, formamide and diiodomethane) were recorded. Surface morphology ($n = 3$) was analyzed with the use of scanning electron microscopy (SEM). The data was statistically analyzed using the two-way analysis of variance (ANOVA) ($\alpha = 0.05$).

Results. Neither 2.5% P-Chi nor 0.5 mol/L EDC influenced the dentin surface wettability ($p > 0.05$). Surface free energy decreased in the eroded substrate after biomodification with EDC ($p < 0.05$). Biomodification with P-Chi demineralized the dentin surface and increased the dentin tubule embouchure.

Conclusions. It can be concluded that 2.5% P-Chi and 0.5 mol/L EDC did not impact the surface wettability of the eroded dentin. However, EDC promoted lower surface free energy, while P-Chi altered surface morphology, causing demineralization and the opening of dentin tubules.

Keywords: chitosan, carbodiimides, tooth erosion

Highlights

- Dental erosion degrades the organic matrix and compromises the quality of the hybrid layer in adhesive restorations.
- Treatment with 2.5% phosphorylated (P-Chi) and 0.5 mol/L carbodiimide (EDC) did not alter the dentin surface wettability, but influenced other surface properties.
- EDC lowered surface free energy, while P-Chi induced demineralization and opened dentin tubules, which might improve adhesion.

Introduction

Dental erosion can result from the prolonged contact of dental tissues with the acidic substances originating from intrinsic factors, extrinsic factors, or a combination of both.[1–3] It is characterized by a chemical process that does not involve bacterial action. From a clinical point of view, this process is defined by the progressive and irreversible loss of the tooth structure through mineral dissolution, culminating in tissue demineralization.[4] Depending on the severity of the lesion, erosion may lead to dental hypersensitivity (DH),[5,6] as well as functional and esthetic damage.

The state of insecurity experienced during the coronavirus disease 2019 (COVID-19) pandemic, mainly due to stress, caused muscular hyperactivity, and the exacerbation of bruxism and temporomandibular disorders (TMD),[7] increasing their prevalence not only in adults, but also in children and adolescents.[8] Bruxism causes a type of pathological wear of the tooth structure due to the clenching and/or grinding of the teeth, and it is another condition that can aggravate the effects of dental erosion. Oral health conditions, such as TMD, bruxism and/or dental erosion, can significantly affect an individual's oral health-related quality of life (OHRQoL). This may have a negative impact on the physical, social and psychological aspects of a person's overall well-being.[9]

What also affects OHRQoL is DH, which is often caused by the exposure of dentin due to erosion, especially in younger patients.[10] It is recommended to adopt healthier dietary practices, including reducing or avoiding acidic foods and beverages, as well as destructive parafunctional behaviors, such as tooth clenching and/or grinding, which can lead to the erosive loss of the tooth structure, and should be discouraged in order to prevent or relieve DH.[11]

Dental erosion causes damage to the tooth structure, leading not only to mineral dissolution, but also to the enzymatic degradation of collagen. This can affect the quality of the collagen network of the hybrid layer, which is a major challenge to overcome in adhesive dentistry,[12,13] as it impacts the long-term integrity of the dentin-bonded interfaces.[14] It directly determines the success of composite restorations, which are the materials of choice in dental practice, being based on minimally invasive approaches,[15–17] and being resistant to erosion from intrinsic and extrinsic acids.[18]

To improve the quality and longevity of adhesive restorations, various studies have evaluated the use of biopolymers, such as chitosan and carbodiimide, to increase the number of cross-links between collagen fibers and neutralize matrix metalloproteinases (MMPs),[19–21] thereby increasing the resistance of the water-rich collagen matrix to enzymatic degradation and preserving the integrity of the hybrid layer.[22,23]

Cross-linking with the use of biomedical agents is one of the strategies used in dentin biomodification.[24–26] Chitosan is a biopolymer obtained by the alkaline deacetylation of chitin; it can be used as a biomodifier increasing the mechanical strength of collagen, which is the main component of dentin, and the resistance of collagen fibrils, which are used as a support to form adhesive interfaces, to hydrolytic and enzymatic degradation.[22,23,27–29] Chitosan is biocompatible, with its low tissue toxicity, potent antimicrobial activity and chelating capacity.[30–32] Dentin treatment with chitosan increases immediate bond strength and improves adhesive infiltration in healthy dentin.[20,33,34]

Chitosan is an important natural polymer used not only in medicine, but also in dentistry, having a wide range of applications.[35] It is currently employed for tissue engineering scaffolds to regenerate the dentin–pulp complex,[36] and bone regeneration due to its biocompatibility, biodegradability, osteoconductivity, and affinity for biomolecules.[37] It can also be added to dental materials, such as glass ionomer cements, which may increase cell viability,[38] chitosan-based drugs in the endodontic treatment of root canals due to its anti-inflammatory, antifungal and antiseptic properties,[39] and the experimental solutions of mouthwashes, which could reduce dentin erosion.[40,41]

The incorporation of new chitosan derivatives by using chemical products, known as biomodification, has been investigated. Biomodification does not alter the functional backbone of chitosan, and retains the original physicochemical and biochemical properties of the biomodifier while providing a matrix with new properties with respect to the added products.[42] Phosphorylated chitosan (P-Chi) derivatives are promising inducers of accelerated

resistance,[43] as they induce calcium phosphate mineral deposition on the partially demineralized dentin surfaces, resulting in low interfacial energy and facilitating dentin surface remineralization.[44] In addition to their proven ability to remineralize the dentin surface, P-Chi derivatives have a high antimicrobial potential.[45]

Carbodiimide (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, or EDC) is an isomeric cyanamide that can aggregate amino acids into peptides without incorporating other groups during the binding process. Carbodiimide has low cytotoxicity, and its mechanism of action involves the formation of covalent peptide bonds between proteins to activate the carboxy-free groups in glutamic acid and aspartic acid present in protein molecules.[46–48] These covalent peptide bonds generate O-acylisourea, which reacts with the epsilon amino group of lysine or hydroxylysine present in the nearest polypeptide chain to form covalent amide bonds,[49,50] resulting in urea as the only residual product, which is easily disposed of with water. These chemical interactions provide EDC with increased collagen strength and assist in the inhibition of protease activity.[33,44]

Dentin treatment with 0.5 mol/L EDC can prevent the degradation of the resin–dentin adhesive interface for up to 12 months, especially when the biomodifier is applied for 60 s, which prevents the degradation of collagen by MMPs.[49] Cadenaro et al. demonstrated that EDC-treated dentin collagen denatured at higher temperatures than the untreated control at the concentrations tested (0.5 M and 1.0 M) during immersion for 10 min or longer.[51] This indirectly indicates a more resistant and highly cross-linked collagen network. Another advantage of EDC is that this non-specific agent acts on a broad spectrum of collagenase-like enzymes (MMPs, cathepsins and others), thus eliminating the need to use different types of agents for each of these enzymes.[12,23,25]

Given the properties of the P-Chi and EDC solutions, they can be used to improve the biomechanical and biochemical properties of dentin, thereby reducing the biodegradation of this tissue and maintaining bond strength over time.[20,22,26,42,52] The aim of the present study was to evaluate the effect of 2.5% P-Chi and 0.5 mol/L EDC on the surface wettability, surface free energy and surface morphology of the eroded dentin.

The null hypothesis tested was that the biomodification of the eroded dentin with 2.5% P-Chi or 0.5 mol/L EDC would not affect the surface wettability, surface free energy or surface morphology of the eroded dentin.

Material and methods

Experimental design

The factors studied were the dentin substrate at 2 levels (sound or eroded) and surface treatment at 3 levels (2.5% P-Chi, 0.5 mol/L EDC or no biomodification (control)). The experimental sample consisted of 144 bovine incisors from the meat-processing industry that would otherwise be discarded. The response variables were as follows: surface wettability, measured as the contact angle between the adhesive system and the dentin surface (n = 12) – quantitative variable; surface free energy, measured as the contact angle between the dentin surface and 3 organic solvents of different polarities (n = 3) – quantitative variable; and surface morphology, analyzed by means of scanning electron microscopy (SEM) (n = 3) – qualitative variable. A flowchart of the study is presented in Fig. 1.

Tooth selection

Bovine incisors, previously preserved in distilled water, were examined microscopically under a magnifying glass (Leica Microsystems, Wetzlar, Germany) at ×20 magnification. Incisors without fracture lines or crown-deep cracks were selected for the study (N = 144).

Sample preparation

The teeth were sectioned transversely at the cemento-enamel junction (CEJ) to separate the crowns from the roots using a double-faced diamond disk (15HC 11-4244; Buehler, Lake Bluff, USA) mounted on a low-speed handpiece (IsoMet® 1000; Buehler). The crowns were then sectioned in the mesial-distal direction, providing 2 hemisections of each crown (buccal and palatal). A dentin fragment with dimensions of 6.0 mm × 6.0 mm × 2.5 mm was obtained from the vestibular hemi-section for all analyses.

The dentin fragments were fixed in Teflon® matrices, using fused wax (Kota, São Paulo, Brazil), with the enamel surface downward. The dentin surface was then polished with Arotec APL-4 polyester (Arotec, Cotia, Brazil), water-cooled and polished with #180–320-grit sandpaper (Hermes Abrasives, Virginia Beach, USA) to adjust the fragment size. Each dentin specimen was subsequently polished with #1,200 grit sandpaper (Hermes Abrasives) for 10 s to flatten the dentin surface. Finally, the specimens were polished with 0.3-micrometer alumina paste (Arotec) on the polishing felt (ATM, Altenkirchen, Germany) for 5 s.

Erosion lesion formation

A protocol established by Vanuspong et al. was used to induce the formation of erosion lesions.[53] Each dentin specimen from a group of 72 (half of the specimens) was immersed in 20 mL of 0.3% citric acid (pH 3.2) and placed on a shaker table (CT-155; CIENTEC Equipamentos Científicos, Belo Horizonte, Brazil), where it remained under constant stirring at 50 rpm for 2 h. Afterward,

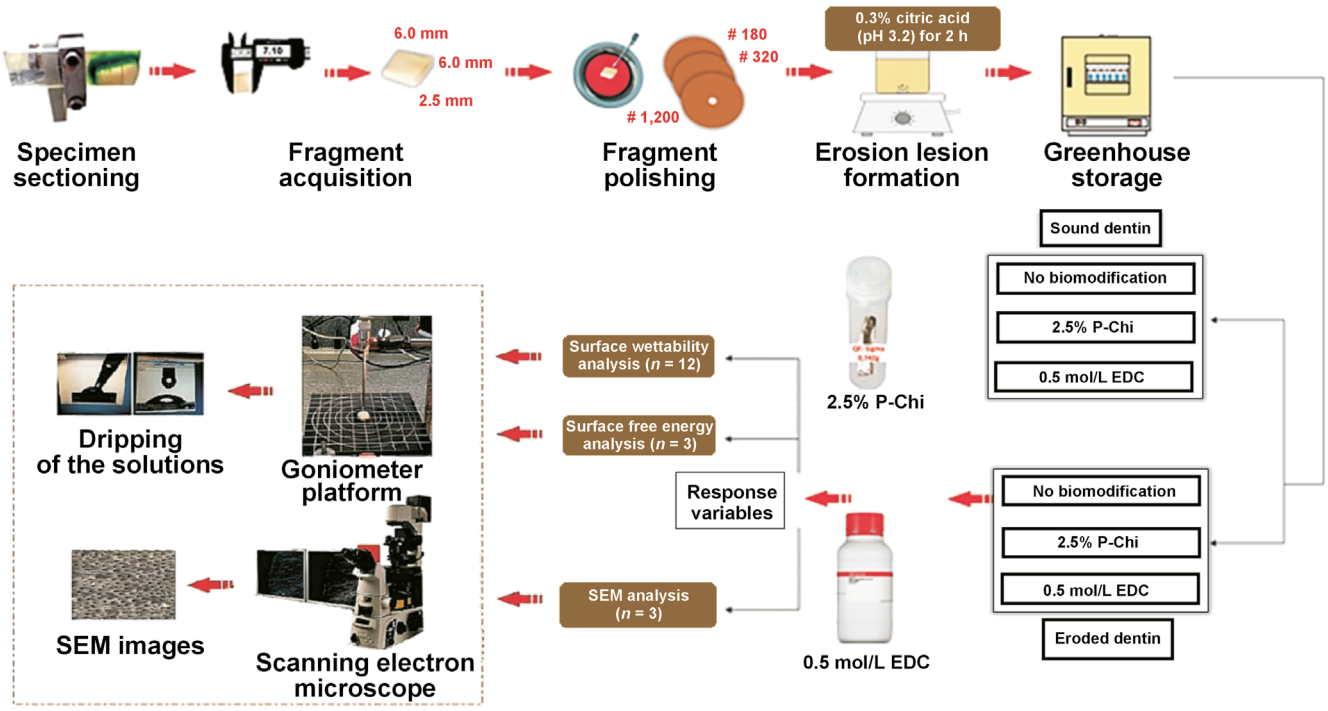

Fig. 1. Flowchart of the study

P-Chi – phosphorylated chitosan; EDC – carbodiimide; SEM – scanning electron microscopy.

the specimens were washed with distilled water, stored individually in Eppendorf tubes containing artificial saliva and kept in an oven at 37°C for 24 h. Artificial saliva consisted of methylparaben (2.0 g), sodium carboxymethylcellulose (10.0 g), KCl (0.625 g), $MgCl_2 \cdot 6H_2O$ (0.059 g), $CaCl_2 \cdot 2H_2O$ (0.166 g), K_2HPO_4 (0.804 g), and KH_2PO_4 (0.326 g) in 1,000 mL of distilled water, according to the protocol described by McKnight-Hanes and Whitford,[54] and modified by Amaechi et al.[55]

Experimental groups

A total of 144 dentin specimens were randomly divided into 6 groups (n = 12), with 24 specimens per group: sound dentin – no biomodification; sound dentin – P-Chi; sound dentin – EDC; eroded dentin – no biomodification; eroded dentin – P-Chi; and eroded dentin – EDC.

Dentin surface treatment with P-Chi

Dentin surface biomodification with P-Chi was carried out according to the procedure described by Wang and Liu.[43] First, the P-Chi solution was prepared by adding 2.5 g of low-molecular-weight chitosan (Sigma-Aldrich, Saint Louis, USA) (75–85% deacetylation), 5 g of urea and 10 mL of phosphoric acid to 40 mL of dimethylformamide (DMF). The mixture was stirred at 150°C for 1 h and filtered. The precipitate was thoroughly washed with distilled water and anhydrous ethanol, and dried under vacuum. The mixture was slowly added to 100 mL of a 1% acetic acid solution under magnetic stirring (Marconi Equipment Laboratories, Piracicaba, Brazil) for 1 h, which was sufficient to solubilize the polysaccharide. Next, 20 µL of 2.5% P-Chi was applied to the dentin surface with a micropipette for 60 s. The surface was then dried with absorbent paper.

Dentin surface treatment with the EDC-HCl solution

The EDC-HCl solution (Sigma-Aldrich) was prepared immediately before use. To obtain an EDC concentration of 0.5 mol/L, 2.3 mg of EDC-HCl was diluted in 230 µL of Milli-Q® water to give a solution with pH 7.34. Then, 20 µL of 0.5 mol/L EDC was applied to the dentin surface for 60 s. The surface was then washed with distilled water for 15 s and dried with absorbent paper.

Surface wettability analysis

Dentin wettability was determined by measuring the contact angle (Θ), using the sessile drop method with a goniometer (OCA 20; DataPhysics Instruments, Filderstadt, Germany). Each specimen was positioned on a movable platform with leveling screws. A 20-microliter drop of an adhesive (Scotchbond™ Universal adhesive; 3M Germany, Neuss, Germany) was then placed onto the specimen and the drop image of the dentin surface was captured for 2 min at intervals of 1 ms, using a lighting system equipped with a tungsten lamp and a charge-coupled device (CCD) camera. A microcomputer processed the image, and the tangent formed between the drop and the

surface was adjusted to determine the Θ values by using the SCA20 software (DataPhysics Instruments). The Θ values were analyzed using the SCA20 program. All procedures involving the Θ measurements were carried out indoors at a controlled ambient temperature of 25 ±1°C.

Surface free energy analysis

Three liquids of different polarities were used to analyze surface free energy: water (a polar universal solvent); formamide (of intermediate polarity); and diiodomethane (highly apolar).

The values for formamide and diiodomethane were used to calculate surface free energy and its components. However, only the water contact angle values were required to explain the surface state.

The contact angle was measured with a goniometer (OCA 20; DataPhysics Instruments), using the sessile drop method. Each sample was positioned on a movable platform with leveling screws. A 20-microliter drop of each liquid (water, formamide or diiodomethane) was then dropped onto the specimen. In the case of formamide and diiodomethane, a micropipette was used to deliver the drop onto the dentin surface; in the case of water, the drop was delivered directly from the goniometer needle. Surface free energy (γS) was calculated from the Θ values regarding the contact angles between the liquids of different polarities and the solid surface. The Owens–Wendt 1969 formula was used to separate the total surface energy into its disperse (d) and polar (p) components (Equation 1):

$$\gamma_L(1+\cos\theta) = 2(\gamma_L^d \gamma_S^d)^{1/2} + 2(\gamma_L^p \gamma_S^p)^{1/2} \quad (1)$$

For each liquid with γL, and its dispersive (γL^d) and polar (γL^p) components, Θ was measured between the liquid itself and the sample. The γS value is obtained as the sum of its dispersive (γS^d) and polar (γS^p) components.

Specimen preparation for SEM

The previously prepared specimens (n = 3) were placed in Eppendorf tubes containing distilled water, and prepared according to the following protocol: ultrasonic cleaning (Ultrasonic Cleaner T-1449-D; Odontobrás, Ribeirão Preto, Brazil) for 10 min; drying with absorbent paper; immersion in a 2.5% glutaraldehyde solution buffered with a 0.1 M sodium cacodylate solution, pH 7.4 (Merck, Darmstadt, Germany), at 4°C for 12 h; washing with distilled water for 3 min, followed by immersion in distilled water for 1 h, changing the water every 20 min; and dehydration in the ascending gradations of ethanol (Labsynth Products Laboratories, Diadema, Brazil) – 25% (20 min), 50% (20 min), 75% (20 min), 95% (30 min), and 100% (60 min). After dehydration, the specimens were immersed in the hexamethyldisilazane (HMDS) solution (Merck) for 10 min for chemical drying. All procedures were performed under a fume hood. After drying, the specimens were fixed in carbon double-sided tape stubs and coated with gold in a vacuum metallization apparatus (SDC 050; Bal-Tec, Balzers, Liechtenstein). The specimens were examined under a scanning electron microscope (XL30 FEG; Philips, Eindhoven, the Netherlands) at the Chemistry Laboratory of the Faculty of Philosophy, Sciences and Letters, University of São Paulo, Ribeirão Preto, Brazil.

The entire surface of the specimens was scanned, and the most representative area of each group was photographed at a single magnification of ×1,500.

Statistical analysis

The Θ values obtained during the surface wettability analysis were subjected to the Kolmogorov–Smirnov normality test, and the data was shown to be normally distributed. The data was then subjected to the analysis of variance (ANOVA) for 2 factors – surface treatment and the dentin surface, with a significance level of 5%. Statistical analysis was performed using the SPSS for Windows, v. 12.0 (SPSS Inc., Chicago, USA).

Results

Table 1 shows the lowest contact angle values obtained based on the surface wettability analysis. ANOVA did not reveal any statistically significant differences between various kinds of treatment ($p > 0.05$). However, biomodification with 0.5 mol/L EDC reduced surface free energy as compared to the control group ($p < 0.05$). The mean surface free energy values ranged from 39.01 mJ/m^2 for the EDC-modified group to 49.23 mJ/m^2 for the control group (Table 2).

Figure 2 shows a schematic representation of the contact angle measurements.

Figure 3 shows the SEM images of the sound and eroded dentin after biomodification, and with no biomodification (control). The sound dentin showed a smear layer and the dentin tubules occluded by peritubular dentin deposits, as well as irregular mineral deposition in intertubular

Table 1. Average values of the smallest contact angle (Θ) presented by each group [°]

Surface treatment	Dentin surface	
	sound	eroded
No biomodification	26.15 ±2.88^{aB}	27.83 ±3.40^{aB}
2.5% P-Chi	33.93 ±2.28^{aA}	36.17 ±3.70^{aA}
0.5 mol/L EDC	26.77 ±3.24^{aAB}	32.89 ±3.84^{aAB}

Data presented as mean ± standard deviation (*M* ±*SD*).
Lowercase letters in superscript indicate differences between various kinds of surface treatment, while uppercase letters in superscript indicate differences between dentin surfaces.

Table 2. Average values of the contact angle (Θ) created by different levels of energy [mJ/m^2]

Surface treatment	Dentin surface							
	sound				eroded			
	water	γS	γS^d	γS^p	water	γS	γS^d	γS^p
No biomodification	40.37 ±9.21	49.23 ±0.95^{aA}	32.82 ±0.99^{aA}	16.41 ±1.02^{bA}	35.59 ±9.80	46.12 ±2.70^{aA}	20.63 ±2.06^{bA}	25.49 ±2.96^{aA}
2.5% P-Chi	55.52 ±3.71	42.94 ±3.40^{aA}	20.94 ±5.68^{aA}	22.00 ±2.89^{aA}	45.49 ±6.18	45.24 ±1.65^{aA}	13.18 ±4.87^{aA}	32.06 ±4.27^{aA}
0.5 mol/L EDC	42.83 ±11.65	48.52 ±1.69^{aA}	28.32 ±2.79^{aA}	20.20 ±2.29^{aA}	59.36 ±14.29	39.01 ±2.15^{bA}	23.91 ±1.66^{aA}	15.10 ±1.30^{aB}

Data presented as *M* ±*SD*.
The γS value is obtained as the sum of its dispersive (γS^d) and polar (γS^p) components.
Lowercase letters in superscript indicate differences between dentin surfaces, while uppercase letters in superscript indicate differences between various kinds of surface treatment.

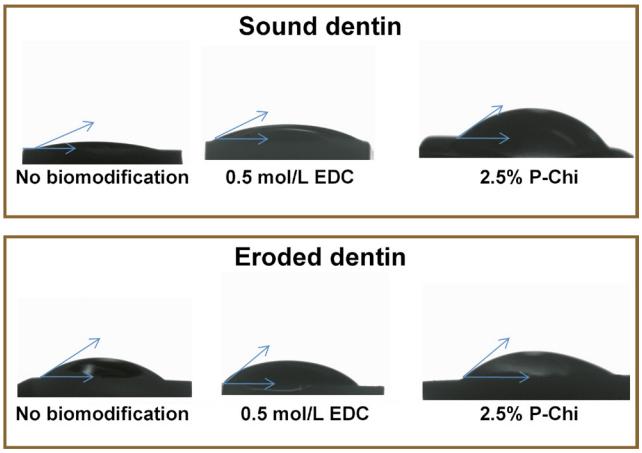

Fig. 2. Representative diagram of the contact angle measurements after surface biomodification

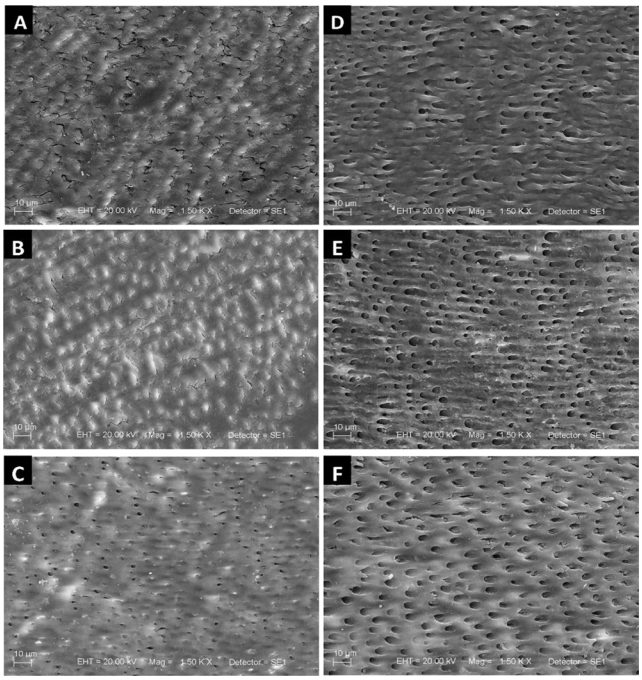

Fig. 3. Scanning electron microscopy (SEM) images of the sound (A, B and C) and eroded (D, E and F) dentin

A, D – no biomodification (control); B, E – 0.5 mol/L EDC; C, F – 2.5% P-Chi.

dentin (sclerotic molds) (Fig. 3A). After biomodification with EDC, dentin tubules remained obliterated (Fig. 3B). Biomodification with P-Chi resulted in partially visible dentin tubules (Fig. 3C). The eroded dentin showed a demineralized organic matrix surface, with no smear layer and open dentin tubules (Fig. 3D). Surface treatment with EDC promoted a demineralization pattern similar to that of the non-biomodified group (control), but dentin tubule embedding was slightly increased (Fig. 3E). Surface treatment with P-Chi induced surface demineralization and slightly increased dentin tubule embedding, possibly due to greater peritubular dentin removal (Fig. 3F).

Discussion

The use of the chitosan solution has shown favorable results in remineralizing the exposed structure of the demineralized dentin[30] and appears to be effective due to its ability to cross-link with dentin type I collagen, which provides protection against enzymatic degradation.[31] Similarly, biomodification with EDC improves the collagen structure and adhesion to it by inactivating dentin MMPs.[25]

Chitosan and EDC are biopolymers widely studied in dentistry, but there are also other natural agents with applications in this field, namely, the propolis extract with cancer-selective toxicity and an anti-inflammatory effect on tongue cancer cells,[56] or as a promising substitute for synthetic remineralizing and antibacterial agents, acting on deep carious dentin,[57] as well as platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) as scaffolds in the process of tissue regeneration, the treatment of intrabony defects and regenerative endodontic treatment.[58] In addition, in organic toothpaste formulations, it is possible to use hectorite (natural clay) as an emulsifier because of its excellent absorption capacity, the *Commiphora myrrha* resin extract that functions as a flavoring agent and a natural antiseptic, among many other natural ingredients, such as *Stevia rebaudiana*, erythritol or xylitol, which have no associated adverse events.[59]

The biomodification of the dentin matrix improves its biochemical and biomechanical properties, with benefits

for preventive and restorative treatment.[26,46] In the present study, the analyzed biomodifiers did not affect the surface wettability of the eroded dentin. This result is in agreement with a study by Ururahy et al., in which no statistically significant differences in wettability were observed after biomodification with chitosan at different concentrations,[60] but partially different from the observations of Curylofo-Zotti et al.[61] In the latter study, when chitosan was used, there was a change in the wettability of the remaining dentin after the selective removal of carious lesions with an erbium-doped yttrium aluminum garnet (Er:YAG) laser, whereas with EDC, there was no change in the dentin wettability.[61] Rehumidification without affecting the wettability of the dentin surface is favorable, since adhesion depends on the flow of the adhesive system on the surface. In this way, we would achieve the positive effects of biomodification (antimicrobial and chelating properties) without losing adhesion.

In terms of surface free energy, it was demonstrated that surface biomodification with 0.5 mol/L EDC promoted a decrease in free energy as compared to the unmodified surface. The total surface free energy is the sum of its disperse and polar components. Changes in these components result in different interactions between the surface and the liquid (biomodifier). The dispersive component favors London-type interactions (dipole-induced or Van der Walls forces) between non-polar molecules, whereas the polar component favors electrostatic, metallic and dipole–dipole interactions between polar molecules.[62] Therefore, these intermolecular interactions, in addition to anion co-adsorption and hydrogen bonding between the surface and the protein, are important factors contributing to the overall interaction.[62,63] The higher the surface free energy, the higher the surface propensity to chemical reactions. In terms of biological environment, high surface free energy values imply a high surface propensity to bind biomolecules.

Surface free energy is related to the chemical groups present on the surface of the sample and, consequently, to the nature of the surface molecular interactions. The polar and non-polar groups of proteins are expected to interact selectively with different surfaces (sound and eroded, modified or not). In the present study, the decrease in surface free energy promoted by biomodification with 0.5 mol/L EDC was due to a decrease in the polar component and a slight increase in the disperse component, indicating an increase in the surface affinity, which was caused by non-polar substances after biomodification with EDC. From a chemical point of view, the fact that EDC showed a higher affinity in the eroded substrate than in the sound substrate could be related to the hydrogen bonding interactions between EDC and the eroded substrate. The treatment of the dentin substrate with diluted acid (in this case, citric acid) promotes the acidic hydrolysis of collagen molecules into amino acid residues, generating amino (-NH2) and carboxyl (-COOH) groups that are hydrogen bond donors to the nitrogen atom of the tertiary amine of the EDC structure. Consequently, the 'non-polar' (less polar) moiety of EDC would be exposed to the substrate surface, thus justifying the decrease in surface free energy by decreasing its polar component and increasing it disperse component. Therefore, the mechanism of action of EDC is expected to involve collagen and occur via intermolecular interactions.

Regarding surface biomodification with 2.5% P-Chi, there is a strong ion–ion interaction between the hydroxyapatite phosphate anions and the cationic moiety of P-Chi (NH2+ group), which is protonated when chitosan is solubilized in acetic acid. Consequently, the polar moiety of the P-Chi structure would remain on the substrate surface, which from a chemical point of view would justify the lack of the influence of 2.5% P-Chi on surface free energy. Therefore, the mechanism of action of P-Chi is via ionic interactions and must involve hydroxyapatite. Based on this explanation, the 2.5% P-Chi treatment was more efficient than the 0.5 mol/L EDC treatment in terms of both surface wettability and surface free energy.

The SEM images showed that the 2.5% P-Chi treatment was more effective in removing the smear layer and increasing the diameter of dentin tubules. This superficial modification is favorable, since chitosan has antimicrobial activity and could contribute to tubule antisepsis in addition to facilitating the flow of the adhesive system.

Limitations

Although the texture and fractal dimension analyses (TA and FD, respectively) have been used in dentistry to evaluate the properties of the surrounding tissues, as well as the incorporated materials,[64,65] the authors of the present study did not have the possibility to use these and other methods of analysis besides SEM images. Another limitation is that, even though the surface wettability, surface free energy and surface morphology analyses were conducted, a long-term bond strength test was not performed. Therefore, it is suggested that further studies be conducted using adhesive bond tests, along with TA and FD, with a long-term follow-up to better evaluate the bond strength with these biomodifiers.

Conclusions

It can be concluded that 2.5% P-Chi and 0.5 mol/L EDC did not impact the surface wettability of the eroded dentin. However, EDC promoted lower surface free energy, while P-Chi altered surface morphology, causing demineralization and the opening of dentin tubules.

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.

ORCID iDs

Mirian Saavedra Lopes Ururahy https://orcid.org/0000-0003-3600-7899
Erick Silva Barbosa https://orcid.org/0000-0002-7346-7010
Rafaela Manente https://orcid.org/0000-0002-1740-6192
Ana Paula Ramos https://orcid.org/0000-0001-6200-8989
Antônio Eduardo Miller Crotti https://orcid.org/0000-0002-1730-1729
Silmara Aparecida Milori Corona https://orcid.org/0000-0002-1733-3472

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Original paper

Effects of processing techniques of monolithic lithium disilicate ceramic on wear resistance against zirconia antagonist

Anselmo Agostinho Simionato[B,D], Alessandra de Sousa Ramos[B], Olívia Breda Moss[B,D], Adriana Cláudia Lapria Faria[C–F], Renata Cristina Silveira Rodrigues[A,C,E,F], Ricardo Faria Ribeiro[A,E,F]

School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):285–292

Address for correspondence
Renata Cristina Silveira Rodrigues
E-mail: renata@forp.usp.br

Funding sources
The study was financially supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brazil (finance code 001), which provided funding for Anselmo Agostinho Simionato's doctoral scholarship and Olívia Breda Moss' master's scholarship. In addition, the study was funded by the Oral Rehabilitation Graduate Program. The National Council for Scientific and Technological Development (CNPq) allocated funds through the Program for Initiation in Basic Research (PIBIC) for Alessandra de Sousa Ramos. The materials necessary for the study were provided by Odontomega (Ribeirão Preto, Brazil).

Conflict of interest
None declared

Acknowledgements
None declared

Received on August 2, 2023
Reviewed on August 22, 2023
Accepted on September 5, 2023

Published online on April 29, 2025

Cite as
Simionato AA, Ramos AS, Moss OB, Faria ACL, Rodrigues RCS, Ribeiro RF. Effects of processing techniques of monolithic lithium disilicate ceramic on wear resistance against zirconia antagonist. *Dent Med Probl.* 2025;62(2):285–292. doi:10.17219/dmp/171899

DOI
10.17219/dmp/171899

Abstract

Background. The durability of the materials used in the prosthesis is a determining factor for the success of the rehabilitation.

Objectives. The aim of the study was to evaluate the wear resistance of monolithic lithium disilicate ceramics processed by heat pressing and computer-aided design/computer-aided manufacturing (CAD/CAM) methods, with a leucite-reinforced feldspathic ceramic processed by CAD/CAM serving as a control group.

Material and methods. Monolithic lithium disilicate ceramic samples, processed as CAD/CAM milled blocks or heat-pressed ingots, were tested against flat zirconia antagonists. A CAD/CAM leucite-reinforced feldspathic ceramic was used as the control specimen. Conical specimens were made for each group and subjected to thermomechanical cycling with a flat zirconia antagonist. The roughness of the conical and flat specimens was evaluated before and after thermomechanical cycling. The height loss in the conical specimens was also assessed. The data concerning height loss was analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc test. The data obtained from the roughness analysis of the conical and flat samples was evaluated using a linear model of repeated measures and Bonferroni complementary test.

Results. A significant difference was observed in the extent of height loss among the ceramics under consideration. Amber® Mill (AMM) exhibited a lesser extent of height loss in comparison to Rosetta® SP (RSP) ($p = 0.010$) and Rosetta® BM (RBM) ($p = 0.005$), yet it demonstrated congruence with Rosetta® SM (RSM) ($p = 0.525$). Additionally, a significant difference was noted between the initial and final roughness values for both the conical ($p = 0.017$) and flat ($p < 0.05$) samples, with the final roughness values being lower than the initial values.

Conclusions. The attrition between ceramic surfaces and zirconia led to a decrease in superficial roughness (Sa). In the context of lithium disilicate ceramics, milled ceramics demonstrated superior performance in terms of wear behavior. The tested feldspathic ceramic exhibited a lower wear resistance compared to the milled lithium disilicate ceramics; however, its wear behavior was similar to that of the heat-pressed lithium disilicate ceramic.

Keywords: dental porcelain, computer-aided design, dental restoration wear, dental materials, lithium disilicate

Highlights

- Among the lithium disilicate ceramics, the milled specimens exhibited superior performance in the wear test.
- The tested feldspathic ceramics demonstrated inferior performance as compared to the milled lithium disilicate, but similar performance to the heat-pressed lithium disilicate.
- A reduction in the surface roughness of zirconia was observed following the wear test.

Introduction

Complete dental ceramic restorations have become a component of oral rehabilitation due to their aesthetic properties, durability and biocompatibility.[1] However, the benefits achieved by restoring a dental element with ceramic materials may be overshadowed by potential wear occurring in the natural dentition or in restorations in the opposing teeth.[1,2] Furthermore, few studies have evaluated the wear resistance of these materials, which is an important factor given ceramics' propensity for wear and subsurface crack formation. These cracks can disseminate, resulting in the loss of fragments.[3] Wear is a factor that is related to occlusal stability and, consequently, can influence the clinical success of the restoration.[2,4]

The wear of the materials used in oral rehabilitation when subjected to the forces resulting from dental contact during chewing, swallowing and speaking can result in the flattening of the cusps of posterior teeth and the incisal shortening of anterior teeth, leading to decreased aesthetics and functionality. Consequently, it is important that the materials used in clinical settings exhibit wear characteristics similar to those of dental enamel to ensure optimal long-term outcomes.[5] The wear of a ceramic prosthesis is a continuous and progressive phenomenon, which is related to material properties and structures, such as surface quality and manufacturing, as well as the environment to which the prosthesis is subjected. The environmental factors include the pH value of saliva, chewing habits, the type of food ingested, and the magnitude of the chewing forces.[6]

Wear is a complex phenomenon that is influenced by several factors, including the microstructural characterization of the materials, the environment in which they are inserted, and the interaction of materials in their environments. The consequences of this condition are the loss of dental anatomy and a reduction in the vertical dimension of occlusion. This, in turn, can lead to physiological and pathological disorders, as well as aesthetic and functional impairment of the restorations.[7]

Thus, the present study aims to evaluate the wear resistance of monolithic lithium disilicate ceramics processed using heat press and computer-aided design/computer-aided manufacturing (CAD/CAM) methods. A leucite-reinforced feldspathic ceramic processed by CAD/CAM will serve as a control group. The factors involved in the wear process, such as roughness and height loss, will be evaluated using a thermal and mechanical cycling test against zirconia antagonists. The null hypothesis posits that there are no differences between the ceramics tested.

Material and methods

Preparation of the specimens

The present study employed a pin-on-block design. Conical ceramic specimens were used, with flat zirconia specimens serving as antagonists. The composition and manufacturing method of the materials used in this study are described in Table 1.

For each ceramic type, specimens in conical shape and rounded tip (3.0 mm × 12.0 mm) were fabricated (n = 5 per ceramic type). Firstly, the ceramics for milling were cut using a diamond trephine with a 3-mm internal diameter (Cortag Revolution Tools, Mogi Mirim, Brazil) and an electric motor (Dremel 3000®; Bosch Power Tools B.V., Breda, Netherlands). This instrument was utilized under conditions of abundant water cooling, resulting in the production of 3-mm diameter cylinders. A cylindrical diamond bur was used in conjunction with a dental drill (D700; Dabi Atlante, Ribeirão Preto, Brazil), with an angle fixed at 30 degrees to the long axis of the cylinder to obtain a conical shape. The ceramic cylinder was affixed to a base in the MultiCP 2000 device (Department of Dental Materials and Prosthesis, School of Dentistry of Ribeirão Preto, Brazil) during the milling process. Subsequently, the specimens were polished using specialized ceramic finishing and polishing rubbers (EVE Diapol; Odontomega, Ribeirão Preto, Brazil) to obtain the rounded shape at the tip.

The obtained specimens were duplicated using heavy (Variotime Easy Putty; Heraeus Kulzer, Hanau, Germany) and light (Variotime Light Flow; Heraeus Kulzer) silicone to create organic wax patterns. These patterns were embedded in casting material (IPS PressVEST Speed; Ivoclar Vivadent, Liechtenstein, Germany) and used for the fabrication of pressed ceramic specimens identical to those obtained for CAD/CAM ceramics.

Table 1. Characteristics of the ceramics used in the study

Material	Type (composition [wt%])	Manufacturing technique	Manufacturer
Amber® Mill (AMM)	lithium disilicate (SiO_2 < 65.00; Li_2O < 10.00; other oxides and colorants <25.00)	CAD/CAM	Human-Aid System Supplier (HASS), Gangneung, South Korea
Rosetta® SP (RSP)	lithium disilicate (SiO_2 < 86.00; Li_2O < 15.00; other oxides and colorants <12.00)	heat pressing	
Rosetta® SM (RSM)	lithium disilicate (SiO_2 < 85.00; Li_2O < 13.00; other oxides and colorants <10.00)	CAD/CAM	
Rosetta® BM (RBM)	leucite-reinforced feldspathic ceramic (SiO_2 < 68.00; Al_2O_3 < 22.00; other oxides and colorants <10.00)	CAD/CAM	
Ceramill® ZI	zirconia	CAD/CAM	Amann Girrbach AG, Koblach, Austria

CAD/CAM – computer-aided design/computer-aided manufacturing.

The specimens to be manufactured by heat pressing (Rosetta® SP (RSP); HASS Corp., Gangneung, South Korea) were vacuum-pressed according to the parameters recommended by the manufacturer. They were divested and blasted with glass beads (110-µm grain size and 4 bar/2 bar fine pressure), while Amber® Mill (AMM) (HASS Corp.) and Rosetta® SM (RSM) (HASS Corp.) were crystallized following the manufacturer's guidelines. Due to the characteristics of Rosetta® BM (RBM) (HASS Corp.), it was determined that crystallization was not necessary after milling. The finishing and polishing of the ceramics was performed with the use of diamond polishers (EVE Diapol Kit; Odontomega).

For the fabrication of flat zirconia specimens, zirconia blocks (Ceramill® ZI; Amann Girrbach AG, Koblach, Austria) were cut to the dimensions of 14.4 mm × 14.4 mm × 1.2 mm using a diamond saw (IsoMet 1000; Buehler, Lake Bluff, USA) and sintered (inFire HTC speed; Dentsply Sirona, São Paulo, Brazil). The heating was initiated at a rate of 10°C/min, reaching a plateau at 700°C and holding for 10 min. Subsequently, the heating was continued at 8°C/min until a second plateau was attained at 900°C, following a holding period of 30 min. The heating was then resumed at a rate of 8°C/min until a third plateau was reached at 1450°C, where it was maintained for a duration of 120 min. Subsequently, the temperature was reduced at a rate of 5°C/min to 200°C. The final dimensions of the samples were 12 mm × 12 mm × 1 mm. The polishing process was executed using #280, #400 and #600 sandpaper, as well as diamond polishers. The samples were then embedded in acrylic resin (VIPI FLASH; VIPI, Pirassununga, Brazil) in polyvinyl chloride (PVC) rings with an outer diameter of 25 mm and a height of 25 mm.

Evaluation of roughness

The tip of the conical samples was analyzed with confocal microscopy (Olympus LEXT OLS4000; Olympus Europa Holding GmBH, Hamburg, Germany) before and after wear tests, and surface roughness values of this region were obtained. The surfaces of the flat samples were also analyzed, and it was possible to obtain the roughness values of the region formed by wear and control areas. The magnification used for the analyses was ×5.

Wear test

Zirconia antagonists and conical specimens were positioned on a two-body wear testing machine (Biocycle; Biopdi, São Carlos, Brazil). The conical specimens were affixed to vertical rods within the machine, and they underwent vertical movements with a 20-N load. The flat specimens were fixed to the base, which slid horizontally for a distance of 3 mm. The frequency of these movements was 2 Hz, and they were performed for 300,000 cycles, which represents 1 year of masticatory function at the average human masticatory frequency.[7,8] Concurrently with mechanical cycling, thermal cycles ranging from 5°C to 55°C were performed. The experimental tank reached maximum capacity in 20 s, maintained that level for 60 s and then discharged in 25 s. This resulted in the completion of 734 thermal cycles in each group.

At the beginning and conclusion of the wear tests, the profiles of the conical specimens were traced using a profile projector (Nikon Profile Projector 6C; Nikon, Tokyo, Japan) at ×10 magnification on a transparent sheet. The positioning of the specimens was standardized before and after the tests. The height loss of the conical specimens was measured using a pachymeter with 0.01-mm precision (Mitutoyo Sul Americana Ltd., Suzano, Brazil). The mass loss was determined by calculating the difference between the final and initial values.

Statistical analysis

The data concerning mass loss and height loss was assessed using the Shapiro–Wilk normality test ($p > 0.05$) and analyzed according to one-way analysis of variance (ANOVA) and Tukey's post hoc test. The data obtained from the roughness analysis with confocal microscopy

of the conical and flat samples was analyzed using a linear model of repeated measures and Bonferroni complementary test. The α level used for the comparisons between the groups was set at 5%. The statistical analysis was conducted using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). The statistical power for the between-group comparisons regarding height loss (95%) and roughness (98.8%) was calculated.

Results

The results for height loss and superficial roughness (Sa) evaluations are presented in Table 2, and Fig. 1 and 2. The statistical analysis revealed a significant difference in height loss after thermocycling between the groups tested ($p = 0.003$). Tukey's post hoc test showed that AMM demonstrated a reduced degree of height loss in comparison to RSP ($p = 0.010$) and RBM ($p = 0.005$), and exhibited similarity to RSM ($p = 0.525$).

Visible chipping of specimens was observed in the RSP (1 event) and RBM (1 event) groups. However, these events did not impede the progression of the test. The roughness results of the conical samples before and after wear tests are illustrated in Fig. 2. The statistical analysis revealed that for the conical specimens, roughness values exhibited a statistically significant difference between the groups ($p = 0.001$), between the evaluated times (initial condition × final condition) ($p < 0.05$) and between the group-time interactions ($p = 0.015$). The surface roughness data of the conical specimens indicates a difference between the initial values. Amber® Mill demonstrated a similarity to all specimens ($p > 0.05$), however, RSP, a heat-pressed ceramic, was different from RSM ($p = 0.029$) and RBM ($p = 0.006$), 2 milled ceramics. After thermocycling, a statistically significant difference was detected between AMM and both RSP ($p = 0.005$) and RBM ($p = 0.005$), and similarity was identified between AMM and RSM ($p > 0.05$). A reduction in surface roughness was noted in all groups after thermocycling ($p < 0.05$). A comparison of the mean difference of the conical specimen roughness reveals a discrepancy between the RSP group and all other groups (AMM: $p = 0.001$, RSM: $p = 0.002$ and RBM: $p = 0.025$).

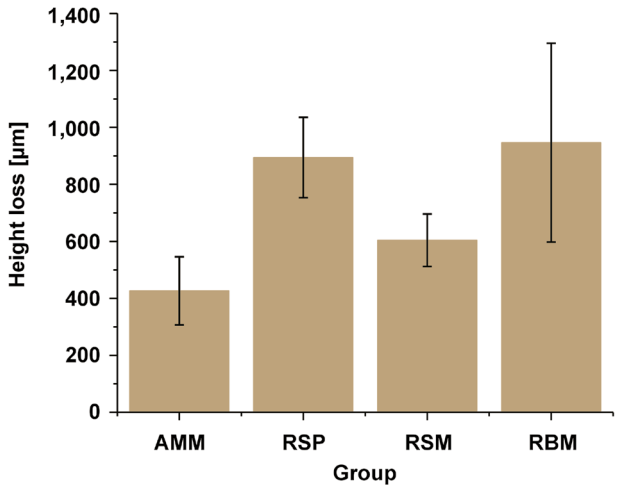

Fig. 1. Wear resistance of the tested ceramics
AMM – Amber® Mill; RSP – Rosetta® SP; RSM – Rosetta® SM; RBM – Rosetta® BM.

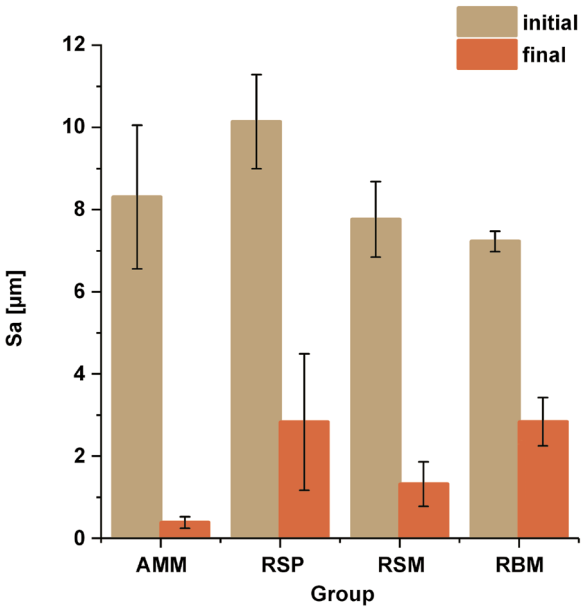

Fig. 2. Initial and final surface roughness (Sa) values of the conical samples

Table 2. Comparison of height loss and surface roughness (Sa) of conical and flat specimens

Specimen type	Variable		Group			
			AMM	RSP	RSM	RBM
Conical	height loss [µm]		426.30 ±119.61^{a}	894.50 ±140.87^{b}	604.00 ±92.17^{ab}	946.40 ±348.97^{b}
	Sa [µm]	initial	8.309 ±1.746^{ab}	10.143 ±1.150^{b}	7.765 ±0.918^{a}	7.230 ±0.248^{a}
		final	0.383 ±0.137^{a}	2.831 ±1.658^{b}	1.318 ±0.544^{ab}	2.832 ±0.681^{b}
Flat	Sa [µm]	control area	1.080 ±0.347^{a}	1.333 ±0.383^{a}	1.544 ±0.523^{a}	1.638 ±0.475^{a}
		wear area	0.282 ±0.066^{a}	0.525 ±0.226^{a}	0.370 ±0.169^{a}	0.239 ±0.159^{a}

Data presented as mean ± standard deviation (*M* ±*SD*). Different uppercase letters represent statistically significant differences between variables in the same row ($p < 0.05$).

The analysis of the data collected on the flat specimens enabled a comparison of a wear area with control areas that exhibited no wear action, as illustrated in Fig. 3. A statistically significant difference was identified between the roughness levels observed in the control area and those measured in the wear area. Specifically, the roughness levels in the wear area were found to be lower than those in the control area ($p < 0.05$). However, no significant difference was detected between the groups ($p > 0.05$). Figure 4 presents the images of the zirconia surface obtained by confocal microscopy following the wear test.

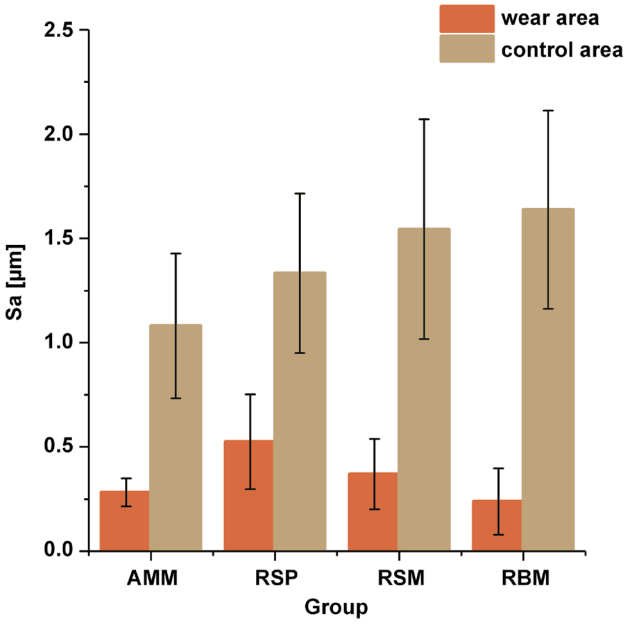

Fig. 3. Surface roughness (Sa) values of the flat samples for the wear and control areas

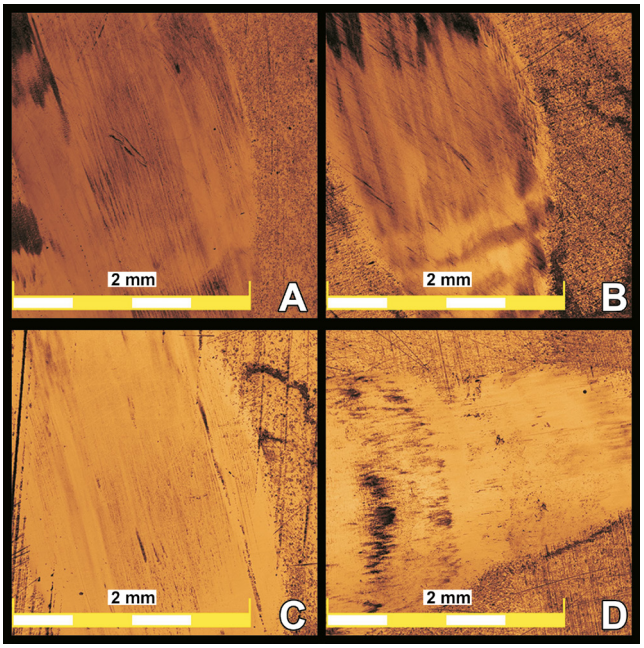

Fig. 4. Images of the zirconia surface obtained by confocal microscopy after the wear test

A. AMM; B. RSP; C. RSM; D. RBM.

Discussion

The present study aimed to evaluate the behavior of lithium disilicate ceramics fabricated with the use of heat press and CAD/CAM methods. A leucite-reinforced feldspathic ceramic was used as a control, and zirconia was utilized as an antagonist material. The conical specimen is a representation of the cuspid occluding on the flat antagonist, and thermomechanical cycling is a representation of the masticatory movement between them. The roughness of the surfaces in contact during the simulated masticatory movement was evaluated before and after the test. The null hypothesis was rejected based on the findings concerning height loss, which revealed a difference between the ceramics tested.

The present study employed a pin-on-block methodology, simulating the contact of the tip of the cuspid, a moment of attrition and disocclusion, added to thermal cycling. The movements performed in this study attempt to simulate the materials and conditions encountered during the application of masticatory forces in localized functional contacts in occlusal rehabilitations. The representation of masticatory forces is methodologically complicated and, in the tests performed in the literature, is described as the continuous cyclic contact between 2 bodies.[8–10] The same difficulty is encountered when simulating the temperature variations occurring inside the oral environment. In this study, cycles ranging from 5°C to 55°C were used to model these variations. A variety of methodologies have been employed in studies on the impact of wear tests on dental ceramics. These methodologies vary with regard to several factors, including the applied force, contact geometry, the role of lubricants, and the number of cycles. This diversity can be attributed to the development and utilization of different cycling devices to replicate the stress conditions of the material within the oral environment.[7,11]

Research has demonstrated that heat-pressed ceramics exhibit superior performance in terms of crown fracture rates, flexural strength, fatigue strength, and survival rate when compared to other materials.[12–14] These properties can be attributed to the enhanced surface finishing achieved through heat pressing. However, the current study demonstrated that CAD/CAM ceramics exhibited superior performance in mitigating height loss, with the exception of RBM. The behavior of the RBM group can be attributed to its composition, which consists of a feldspathic ceramic reinforced by leucite. The wear resistance of lithium disilicate exceeds that of feldspathic ceramics, as indicated by the findings of several studies.[15,16] In the present study, lithium disilicate ceramics, with the exception of RSP, performed better than the leucite-reinforced feldspathic ceramic (RBM). The composition of feldspathic ceramics is predominantly characterized by the glass matrix, making them highly aesthetic and suitable for use in the rehabilitation of anterior teeth.[5,17] However,

the aesthetic characteristics are improved at the expense of the mechanical properties of this material.[5] In the context of wear, crystalline particles with dimensions below 5 μm have a strong influence on surface roughness.[1,7] Lithium disilicate ceramics consist of ultra-fine particles (4 μm) arranged in various orientations.[7,18] This could reflect the wear of the flat samples due to friction between the bodies involved in the wear process and also to changes in the coefficient of friction.[1,7] A similar phenomenon occurs when analyzing ceramics containing leucite, where the size of the particles, their distribution, concentration, and density of the glass matrix influence the wear resistance.[1,16]

Heat-pressed ceramics can present porosities in their structure, a consequence of the manufacturing process. These porosities promote the propagation of microcracks,[3,19] which may underlie the occurrence of chipping in the RSP ceramic group. Chipping in RBM may be associated with the milling process and structural changes that occur in the production process.[14] Chipping of ceramic prostheses is not an atypical event and can be caused by surface failures, which reduce the critical loads that generate larger fractures.[16] Milling performed on CAD/CAM blocks can induce a cascade of events on the surface and in the structure, leading to lateral and radial fractures, chipping, and introduction of residual stress. These phenomena are potential initiators of fracture and consequent failure of the obtained restorations.[14,20] The crystallization process is not necessary for RBM, which is just milled. Crystallization has a positive influence on the presence of microfractures on the ceramic surface when compared to non-crystallized ceramics.[21,22] This process could influence the degradation of the glass matrix and generate more favorable residual stresses at the glass-crystal interface.[22] The influence of these factors on the results obtained in this study is a subject for further investigation, as the lithium disilicate ceramics that underwent this process (AMM and RSM) had smaller height loss values.

One of the wear mechanisms encountered in clinical settings is the two-body wear, defined as the direct contact between the prosthesis and the antagonist, or contact between the tooth and the prosthesis during the process of saliva swallowing or speech. Abrasive wear is a reaction caused by the friction between 2 bodies with distinct surfaces in continuous or dynamic contact. This reaction is evidenced by the plow pattern on the evaluated surface.[1,6] In the present study, the pattern can be visualized in the flat specimens (Fig. 3), indicating frictional wear between the surfaces. The three-body wear occurs when food is chewed and there is dynamic tooth–food–prosthetic contact.[2,6] The literature includes methodologies that use artificial saliva, glycerin and polishing pastes to perform the tests, characterizing the wear between 3 bodies and changing the coefficient of friction.[1,7,8,23] Multiple microcontacts, represented by the roughness of an antagonist body or debris from the abraded surface at the sliding surface, can be associated with an increase in wear intensity.[16] A number of studies have indicated a correlation between wear and material characteristics, compositions, and the presence of external factors such as abrasives and surface roughness.[1,2] In the present study, the wear between 2 bodies was conceptualized. However, even with abundant irrigation of the wear area by the thermomechanical cycling machine, a layer of debris remains between the conical and the flat specimens, which may cause additional wear. Furthermore, the presence of microscopic cracks is indicative of three-body wear.[3,24]

Surface roughness is an important factor to be evaluated. Elevated surface roughness values can lead to a number of changes in the mechanical properties of materials, including the occurrence of caries, food impaction and excessive wear of antagonist and adjacent teeth.[2,25] The obtained results indicate a significant decrease in surface roughness of the conical samples after thermomechanical cycling across all the evaluated groups. The surface characteristics of the material are a significant factor in the initial stages of the wear process. However, upon altering the surface characteristics, the material properties become the determining factor in wear behavior.[26] Roughness analyses performed using confocal laser scanning microscopy on the flat specimens revealed significant differences between the wear and control areas, with lower roughness values observed in the wear area for all the groups evaluated. Roughness and the coefficient of friction can change depending on the duration of a test and the materials being tested, which may explain the low association between these 2 properties and the amount of wear.[22,26,27] With the methodology employed in this study, there was a decrease in the roughness of conical and flat specimens in all groups after thermocycling.

It is challenging to evaluate the different variables that contribute to enamel wear and the materials involved in a single study. The majority of studies use dental enamel or enamel analog materials as antagonists.[2,3,5,7] Thus, it is intriguing that studies of commercially available materials are performed against various antagonists, thereby ensuring homogeneity in the evaluations. This homogeneity is not guaranteed when using natural dental enamel,[28] as it is typically obtained from multiple donors. In this study, zirconia was utilized as an antagonist to the materials tested. Although the use of zirconia as an antagonist could be regarded as one of the study's limitations, its implementation ensured that the ceramics were exposed to identical test conditions for the assessment of wear. Although this combination is not the most frequent in the buccal cavity, the homogeneity of the antagonist ensured more standardized conditions during the tests. This is in contrast to the conditions present in the mouth, where natural variations in human dental enamel are observed.

This study is subject to other limitations regarding the fractographic interpretation of the materials after testing. Confocal laser scanning microscopy analysis is intended to measure the depth of irregularities in the materials. This analysis does not enable to obtain high-resolution images of their surface, which infers that possible cracks located in the wear areas are interpreted as roughness data.[13,21,23] Another analysis that could be performed to help interpret the obtained values is the evaluation of step height in the antagonist specimens. However, this analysis could not be performed on the flat specimens.

Conclusions

In consideration of the study's limitations, it can be concluded that the attrition between ceramic surfaces and zirconia leads to a reduction in Sa in the occlusal contact area. Furthermore, within the group of lithium disilicate ceramics, milled ceramics demonstrated better results in terms of wear behavior under the conditions tested. The enhanced homogeneity of CAD/CAM blocks for machining has been demonstrated to ensure superior resistance to wear. Therefore, clinicians should consider these materials a priority. The findings of the study indicated that the tested feldspathic ceramic exhibited a lower wear resistance compared to the milled lithium disilicate ceramics. However, its wear behavior was similar to that of the heat-pressed lithium disilicate ceramics.

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.

ORCID iDs

Anselmo Agostinho Simionato https://orcid.org/0000-0002-6913-0001
Alessandra de Sousa Ramos https://orcid.org/0000-0002-0927-7394
Olívia Breda Moss https://orcid.org/0000-0002-1425-2247
Adriana Cláudia Lapria Faria https://orcid.org/0000-0001-6353-1480
Renata Cristina Silveira Rodrigues https://orcid.org/0000-0003-4140-4143
Ricardo Faria Ribeiro https://orcid.org/0000-0003-4211-0542

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Original paper

Evaluating the antibacterial and antibiofilm activity of *Zataria multiflora* in comparison with chlorhexidine, using a tooth model: A preliminary study

Aida Mehdipour[1,2,A,C,D], Raziye Pourreisi[3,B,C], Hossein Amini-Khoei[4,A,D], Saeed Shams[2,A,C–F]

[1] Department of Pediatric Dentistry, Faculty of Dentistry, Qom University of Medical Sciences, Iran
[2] Cellular and Molecular Research Center, Qom University of Medical Sciences, Iran
[3] Student Research Committee, Qom University of Medical Sciences, Iran
[4] Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Iran

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):293–298

Address for correspondence
Saeed Shams
E-mail: sshamsmed@gmail.com

Funding sources
Research Council of Qom University of Medical Sciences, Iran (grant No. 1089).

Conflict of interest
None declared

Acknowledgements
The authors wish to thank the Research Council of Qom University of Medical Sciences, Iran, for approving the project.

Received on March 30, 2022
Reviewed on May 22, 2022
Accepted on June 3, 2022

Published online on April 30, 2025

Cite as
Mehdipour A, Pourreisi R, Amini-Khoei H, Shams S. Evaluating the antibacterial and antibiofilm activity of *Zataria multiflora* in comparison with chlorhexidine, using a tooth model: A preliminary study. *Dent Med Probl.* 2025;62(2):293–298. doi:10.17219/dmp/150652

DOI
10.17219/dmp/150652

Abstract

Background. The formation of biofilm on the tooth surface can lead to the dissolution of the enamel minerals and the onset of tooth decay. Natural compounds may prove to be effective in the prevention of biofilm formation.

Objectives. The present study aimed to evaluate the antibacterial and antibiofilm activity of the *Zataria multiflora* ethanolic extract in comparison with chlorhexidine, using a novel tooth model.

Material and methods. The study used teeth extracted due to orthodontic treatment or impacted wisdom tooth surgery. Saliva was collected from a volunteer 12 h after tooth brushing, before the test, and it was used freshly. The teeth were placed in 5 test tubes containing a broth medium and serial dilutions of the *Z. multiflora* extract (50, 25, 12.5, 6.25, and 3.125 mg/mL). A total of 1 mL of the collected saliva was added to each test tube. The growth of microorganisms in the medium was examined visually and the antibiofilm activity of the plant extract was assessed after 72 h, using a spatula. The results were compared with those of positive and negative controls.

Results. Considerable turbidity was observed in the positive control tube containing a tooth, the culture medium and saliva, indicating that the conditions were favorable for the growth of oral flora. No bacterial growth or biofilm formation were observed in the test tubes containing ≥25 mg/mL of the plant extract.

Conclusions. The study results indicated that the *Z. multiflora* extract had an excellent inhibitory effect against microorganisms and plaque formation in the tooth model, suggesting a suitable substitute for chlorhexidine. However, further studies in this area are recommended.

Keywords: *Zataria multiflora*, dental plaque, biofilm, tooth model, normal oral flora

Highlights

- This preliminary study used a novel tooth model to directly assess the antibacterial and antibiofilm effects of the *Zataria multiflora* extract, a promising plant-based alternative for oral care.
- The *Z. multiflora* extract, at concentrations of 25 mg/mL or higher, demonstrated strong antibacterial activity and the prevention of dental plaque formation, showing no visible plaque on the treated teeth.
- Under conditions mimicking the oral cavity, the effectiveness of *Z. multiflora* was comparable to that of chlorhexidine, suggesting its potential as natural treatment for dental plaque and oral infections.

Introduction

Dental caries, also known as tooth decay, is caused by cariogenic organisms, including streptococci, lactobacilli, etc., present in the biofilm (dental plaque), which ferment dietary carbohydrates to produce acids, leading in turn to the dissolution of the enamel minerals and the onset of tooth decay.[1,2] Among streptococci, the *mutans* group, especially *Streptococcus mutans*, is considered to be the main cause of tooth decay. However, some studies have shown that other bacteria can also be involved in the development of caries.[3] On the other hand, dental plaque can be a reservoir of pathogenic strains of *Helicobacter pylori* and may play an important role in gastrointestinal diseases.[4]

Commercial mouthwashes act as an adjunct to mechanical methods, such as tooth brushing and flossing; they play an important role in reducing the bacterial load, and thus caries.[5] Among antimicrobial agents, chlorhexidine, a biguanide-based chlorophenyl with an extensive antimicrobial activity, has been regarded as the gold standard.[6,7] Unfortunately, the long-term use of chlorhexidine could lead to side effects, such as a decreased salivary flow, the discoloration of the tongue, mouth and teeth, a burning sensation in the mouth, etc.[8] Therefore, taking into account the lower resistance of bacteria to plant essential oils, other agents, such as herbal mouthwashes, have been increasingly studied in recent years.[9–11]

Zataria multiflora, which grows in different parts of Iran, is one of the dicotyledonous plants belonging to the mint family (*Lamiaceae*). The plant contains effective antimicrobial compounds, such as carvacrol, thymol and linalool; however, their amount may vary depending on the plant variety and cultivation area.[12] As the *Z. multiflora* extract is natural and inexpensive, apart from other advantages, it could be considered as a substitute for commercial antiplaque agents.[13,14] Although several reports have investigated the antibacterial and antibiofilm activity of different extracts in vitro, using the microtiter method,[15] the present study was meant to be novel in terms of using a tooth model with a close simulation of the oral cavity, and it aimed to evaluate the antibacterial properties of *Z. multiflora* through assessing the inhibition of plaque formation by normal flora in comparison with commercial chlorhexidine.

Material and methods

Collection of teeth and saliva

The study was reviewed and approved by the Medical Ethics Committee of Qom University of Medical Sciences, Iran (code: IR.MUQ.REC.1399.029), and all procedures were carried out in accordance with the relevant guidelines and regulations of the committee. After providing the necessary explanations to the patients referred to a dental clinic in Qom, and receiving informed written consent from all subjects or their legal guardians, teeth that had to be extracted due to orthodontic treatment or impacted wisdom tooth surgery were collected. Therefore, no additional teeth were extracted from the patients except those that were in the treatment process. The collection of saliva from a volunteer was also based on informed written consent.

A total of 27 healthy human teeth, with no caries, cracks or previous restoration, were collected. After extraction, the teeth were placed in 0.9% physiological saline solution until transferred to the laboratory. For disinfection, all the teeth were kept in 10% formalin solution for 7 days. After that, the remaining soft tissue was removed from the tooth surface with a periodontal curette. Then, the teeth were cleaned using a rubber cap and sterilized in an autoclave at 121°C for 15 min.[16]

Saliva, containing nutrients and a variety of oral microorganisms that could play a significant role in biofilm formation and the development of caries, was collected from a volunteer with no active tooth decay 12 h after tooth brushing, before the test, and used freshly.

Preparation of the plant extract

To prepare the extract, the *Z. multiflora* plant was purchased from an herbal medicine shop in Qom, Iran. The identification and authentication of the purchased plant were done by the relevant academic staff from the Department of Persian Medicine at Qom University of Medical Sciences. Then, 20 g of the plant powder was dissolved in 100 mL of 96% ethanol solvent (Taghtir Khorasan Co., Mashhad, Iran) and the obtained solution was placed in a dark environment. After 3 days, the

solution was filtered, and the resulting clear solution was poured into a glass plate and again placed in a dark environment for 2–3 days so that ethanol could evaporate. The obtained powder was then stored at 4°C until used for further experiments.

Evaluation of antibacterial and antibiofilm effects

For this purpose, the teeth were placed in 5 test tubes containing 3 mL of the tryptic soy broth (TSB) medium (Ibresco, Karaj, Iran). A total of 3 mL of the plant extract stock solution (100 mg/mL) was added to the 1st tube, and then serial dilutions were made to prepare concentrations of 50, 25, 12.5, 6.25, and 3.125 mg/mL. Afterward, 1 mL of the collected saliva was added to each test tube. Four control tubes containing the following materials were also used: a tooth, the culture medium and saliva (positive control PC1); a tooth, the culture medium, saliva, and 0.2% chlorhexidine (positive control PC2); a tooth, the culture medium and 50 mg/mL of the extract (negative control NC1); and a tooth and the culture medium (negative control NC2). The tubes were incubated at 37°C in a shaker incubator and visually evaluated for the growth of microorganisms at 24, 48 and 72 h. The lowest concentration that inhibited the growth of normal flora was identified as the minimum inhibitory concentration (MIC). Finally, the teeth were removed from the culture medium, and the antibiofilm properties of the used materials were evaluated by determining the presence or absence of dental plaque on the tooth surface with a spatula. Toensure the accuracy of the experiment, it was repeated in 3 groups of 9 teeth.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA), and employing the Kruskal–Wallis test to compare the antibacterial and antibiofilm effects of the *Z. multiflora* extract with regard to different concentrations. A p-value of less than 0.05 was considered as statistically significant.

Results

In daily examinations, considerable turbidity was observed in the positive control tube containing a tooth, the culture medium and saliva (PC1), indicating that the conditions were favorable for the growth of normal oral flora. No bacterial growth or turbidity were observed in the tubes containing 25 and 50 mg/mL of the extract (MIC = 25 mg/mL), while these were visible in the tubes containing other concentrations of the extract ($p = 0.007$). Slight turbidity was also observed in the tube containing chlorhexidine (PC2), indicating a poor growth of microorganisms. No bacterial growth was observed in the negative control tubes (Fig. 1).

The inhibitory activity of the extract against biofilm formation on the tooth surface was evaluated after 72 h, using a spatula. The results showed that the extract at concentrations of 25 and 50 mg/mL also exhibited a significant inhibitory effect against biofilm formation, and no plaque was formed on the tooth surface, similar to the negative control teeth ($p = 0.007$). The extract showed no inhibitory effect on biofilm formation at lower concentrations. Therefore, the MIC of the extract against biofilm formation was determined to be 25 mg/mL. Interestingly and unexpectedly, in the positive control tube PC2, commercial chlorhexidine was not able to completely inhibit biofilm formation as compared to the extract at concentrations of 25 and 50 mg/mL (Fig. 2). All results were similar in the 3 groups. The findings are summarized in Table 1.

Discussion

To date, tooth decay remains a major challenge in public healthcare systems and one of the most common diseases worldwide, experienced by almost everyone during their lifetime. Microorganisms are the crucial contributors involved in the development of dental caries through the fermentation of carbohydrates and the production of acids.[1,3,17]

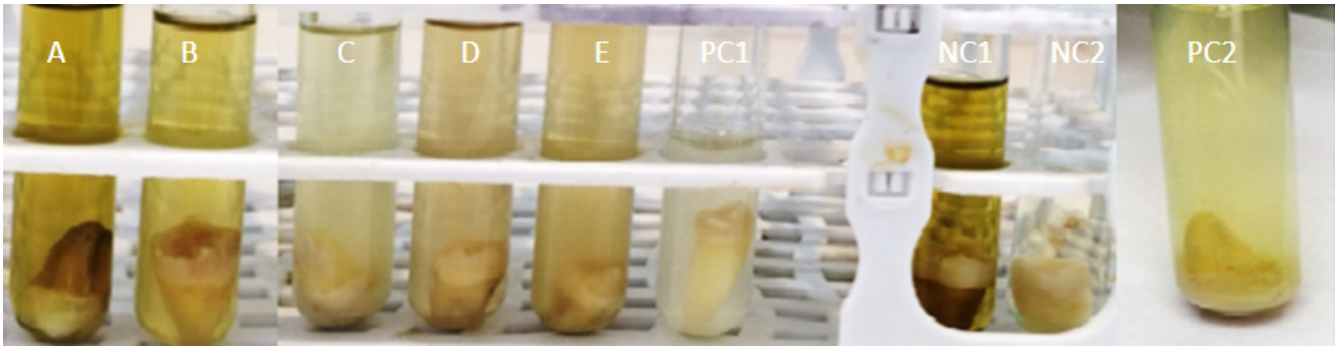

Fig. 1. Test and control tubes after 72 h with regard to microbial growth

A – 50 mg/mL; B – 25 mg/mL; C – 12.5 mg/mL; D – 6.25 mg/mL; E – 3.125 mg/mL; PC – positive control; NC – negative control.

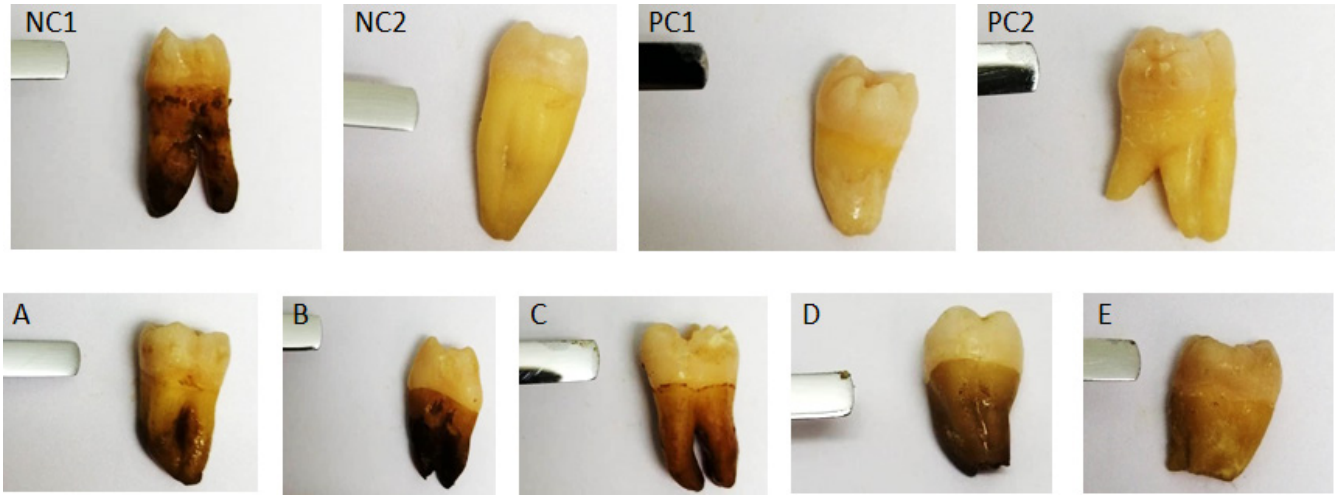

Fig. 2. Investigation of the inhibitory effect on biofilm formation after 72 h

A – 50 mg/mL; B – 25 mg/mL; C – 12.5 mg/mL; D – 6.25 mg/mL; E – 3.125 mg/mL. In pictures C, D and E, the plaque isolated from the tooth surface could be observed on the spatula.

Table 1. Effect of different concentrations of the *Zataria multiflora* extract on the growth of normal flora and biofilm formation after 72 h

Variable	Various extract concentrations [mg/mL]					Controls			
	50	25	12.5	6.25	3.125	PC1	PC2	NC1	NC2
Growth of normal flora	–	–	+	+	+	+	+	–	–
Biofilm formation	–	–	+	+	+	+	+	–	–

Zataria multiflora has been shown to have acceptable inhibitory effects on pathogens, especially antibiotic-resistant bacteria. In a study conducted by Saeidi et al., the antibacterial activity of *Mentha longifolia* L. (ethyl acetate and aqueous extracts) and *Z. multiflora* (a hydroalcoholic extract) against some bacterial species was investigated.[18] The researchers reported that these extracts were able to inhibit the growth of all the tested species.[18] Dadashi et al. showed that *Z. multiflora* might act in vitro against the multidrug-resistant strains of *Klebsiella pneumonia*.[19] The effects of *Z. multiflora* against the metallo-beta-lactamase (MBL)-producing *Pseudomonas aeruginosa* isolates and the antibiotic-resistant *Staphylococcus aureus* strains isolated from food were also reported by Heidary et al.[20] and Soltan Dallal,[21] respectively.

As previously mentioned, no study has been conducted so far to investigate the antibacterial and antibiofilm effects of *Z. multiflora* on a dental model in vitro. In this project, efforts were made to create a close simulation of the oral cavity, which is one of the strongest points of this study. In the present study, it was observed that the *Z. multiflora* extract at a concentration ≥25 mg/mL significantly inhibited the growth of oral microorganisms.

The antimicrobial activity of *Z. multiflora* is due to the fact that the plant is a rich source of oxygenated monoterpenes, especially thymol and carvacrol, which have been well established as excellent antimicrobial agents.[12,14] In a study examining the biological importance of the *Z. multiflora* extract, Saleem et al. showed that thymol was the most important component in the fresh plant, while carvacrol was the most important component in the dried plant.[22] It is known that lipophilic compounds in thymol and carvacrol cause damage to the cell membrane through changing its permeability, and ultimately leading to the lysis of bacterial cells.[23,24]

In the present study, in the positive control tube (PC2), the commercial chlorhexidine mouthwash could not completely inhibit bacterial growth and plaque formation, indicating that chlorhexidine was less effective than the *Z. multiflora* extract, which showed significant antibacterial and antibiofilm activity at concentrations of 25 and 50 mg/mL.

Studies have shown that chlorhexidine plays an important role in preventing plaque formation. For example, Haydari et al. investigated the effect of chlorhexidine (0.06%, 0.12% and 0.2%) on plaque formation and bleeding, as well as its side effects on the modified experimental gingivitis model; the results showed that 0.2% chlorhexidine exhibited a significant inhibitory effect on plaque formation as compared to concentrations of 0.06% and 0.12%.[25]

However, other reports comparing chlorhexidine with herbal mouthwashes, especially *Z. multiflora*, have indicated that herbal mouthwashes also show good effects in terms of microbial control. Jafari et al. examined the effects of a *Z. multiflora* essential oil solution and a chlorhexidine mouthwash on the orthodontic elastic rings contaminated with *S. mutans* in vitro.[26] It was found that 0.5 mg/100 cm^3 solution had good antibacterial properties, and therefore could be used for controlling microbial plaque in orthodontic rings as a substitute for chlorhexidine.[26] In another

report by Aghili et al., the antimicrobial effects of the *Z. multiflora* extract and chlorhexidine on the orthodontic elastomeric ligatures experimentally contaminated with *S. mutans*, *Enterococcus faecalis* and *Candida albicans* were compared.[14] Due to statistically significant differences in the count of viable bacteria before and after disinfection with *Z. multiflora*, they concluded that the plant extract had antibacterial properties and could be used to disinfect orthodontic elastomeric ligatures.[14] Moreover, the inhibitory concentrations of both thymol-based and chlorhexidine mouthwashes with regard to the growth of *Streptococcus* spp. were considered and compared by Khorasani et al.[27] They showed that both types of mouthwash were effective in inhibiting the growth of the studied bacteria; however, the chlorhexidine mouthwash was more potent than the thymol-based mouthwash in inhibiting bacterial growth if diluted.[27]

Limitations

Due to the coronavirus disease 2019 (COVID-19) pandemic, it was not possible to collect saliva from people who had active caries and did not brush their teeth regularly, which may have affected the results. Due to some limitations in our laboratory, certain methods, like atomic force microscopy (AFM) or confocal laser scanning microscopy (CLSM), could not be used to analyze biofilm viability and structure.

Conclusions

This was a preliminary study, designed to use a tooth model. Considering that *Z. multiflora* is one of the native medicinal plants of the tropical regions of Iran, as well as due to its reasonable price and fewer side effects in comparison with chlorhexidine, it is suggested to use this plant extract as a mouthwash or chewing gum. While confirming the findings of other studies regarding the effectiveness of this plant, the results of the present work with the simulation of the oral cavity environment also showed that *Z. multiflora* was comparable with chlorhexidine. However, it is recommended that further studies be performed, involving microbiological analysis, the microscopic evaluation of the structure of the biofilm, and finally the use of the extract in vivo.

Ethics approval and consent to participate

The study was reviewed and approved by the Medical Ethics Committee of Qom University of Medical Sciences, Iran (code: IR.MUQ.REC.1399.029), and all procedures were carried out in accordance with the relevant guidelines and regulations of the committee. All subjects or their legal guardians provided informed written consent before the commencement of the study.

Data availability

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

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Aida Mehdipour https://orcid.org/0000-0002-1456-4267
Raziye Pourreisi https://orcid.org/0000-0001-7231-7734
Hossein Amini-Khoei https://orcid.org/0000-0002-5029-9632
Saeed Shams https://orcid.org/0000-0002-3701-3126

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Original paper

Impact of high-consumption beverages on the color, and surface roughness and microhardness of resin-matrix ceramics

Ebele Adaobi Silva[A,B,D], Anselmo Agostinho Simionato[D,E], Olívia Breda Moss[B,D], Adriana Cláudia Lapria Faria[B,C,E], Renata Cristina Silveira Rodrigues[A,C,E], Ricardo Faria Ribeiro[A,C,E,F]

Department of Dental Materials and Prostheses, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):299–307

Address for correspondence
Renata Cristina Silveira Rodrigues
E-mail: renata@forp.usp.br

Funding sources
Scholarships for E.A. Silva and A.A. Simionato (PhD), as well as for O.B. Moss (Master's degree), funded by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil, within the High Standard Promotion of Graduate Courses program, and the financial support of the Oral Rehabilitation Graduate Program (code 001).

Conflict of interest
None declared

Acknowledgements
The authors thank CAPES, Brazil, for the scholarships, the Oral Rehabilitation Graduate Program for financial support and Singular Implants (Parnamirin, Brazil) for donating the materials.

Received on July 7, 2023
Reviewed on August 13, 2023
Accepted on August 17, 2023

Published online on April 30, 2025

Cite as
Silva EA, Simionato AA, Moss OB, Faria ACL, Rodrigues RCS, Ribeiro RF. Impact of high-consumption beverages on the color, and surface roughness and microhardness of resin-matrix ceramics. *Dent Med Probl.* 2025;62(2):299–307. doi:10.17219/dmp/171320

DOI
10.17219/dmp/171320

Abstract

Background. Resin-matrix ceramics have been developed to combine the high esthetics of ceramics with the mechanical properties of composite resin. The surface changes of these materials when exposed to highly consumed beverages are still not fully elucidated and need further research.

Objectives. The aim of the present study was to evaluate the color stability, and surface roughness and microhardness of a polymer-infiltrated ceramic network (PICN) (VITA ENAMIC® – VE) and a resin nano-ceramic (Ambarino® High-Class – AH), used to obtain computer-aided design/computer-aided manufacturing (CAD/CAM) restorations, after immersion in high-consumption beverages.

Material and methods. A total of 96 specimens were prepared ($n = 48$ per group – VE and AH), further subdivided into 6 groups ($n = 8$) according to the beverage in which they were immersed. The color change (ΔE_{00}), surface roughness (Ra) and microhardness (KHN) measurements were made before and after immersion in the beverages.

Results. The materials presented significant differences in ΔE_{00} when immersed in distilled water ($p = 0.043$), coffee ($p = 0.010$) and red wine ($p < 0.001$). Acceptable values for ΔE_{00} were recorded for distilled water and the energy drink with regard to AH, and for the energy drink and orange juice with regard to VE. Regarding the Ra data, the VE samples showed a difference after immersion in all the tested beverages, while AH differed only when immersed in distilled water and the energy drink. Immersion in the beverages seemed to reduce KHN in the tested materials, although significant differences were detected only in the VE samples from the energy drink ($p < 0.001$) and orange juice ($p < 0.001$) groups.

Conclusions. Based on the results, it can be concluded that the beverages tested may affect the esthetics and surface characteristics of the materials tested.

Keywords: hardness, color, dental materials, organically modified ceramics

Highlights

- Widely consumed beverages, such as coffee, red wine, soda, and orange juice, can significantly compromise the color, and surface roughness and microhardness of resin–matrix ceramics.
- The Ambarino® High-Class resin-matrix dental material showed superior resistance to color and surface roughness changes as compared to VITA ENAMIC®, highlighting the importance of the resin-matrix composition in long-term material stability.
- The degradation of the polymeric matrix in an acidic environment exposes ceramic particles, increasing roughness and susceptibility to pigment absorption, which negatively affects the esthetics and longevity of restorations.
- It is recommended to limit exposure to acidic and pigmented beverages, and to adopt adequate oral hygiene practices to preserve the esthetic and mechanical properties of restorations.
- Future studies should investigate aging methods, the finishing and polishing systems, and the simulation of the oral environment to enhance the understanding and clinical performance of resin–matrix ceramics.

Introduction

The success of any dental material used during the rehabilitation of a missing tooth is directly related to the maintenance of its esthetic and mechanical characteristics over time.[1–3] The oral cavity is an aqueous environment, with variations in pH and the temperature due to the ingestion of different beverages and foods. The materials exposed to such variations can alter their esthetic and mechanical properties. These alterations may occur due to acid substances, which affect the surface morphology of the material, or through the accumulation of pigment; such factors are described as extrinsic.[1,3] Also, the intrinsic characteristics of the material may lead to the failure of the restoration.[1,3–6] Given a high demand from patients for biomimetic dental restorative materials, ceramics and composites have been questioned with regard to the maintenance of their properties for a long time.[6,7] Color or surface changes can limit the esthetics and longevity of restorations.[3]

The use of ceramics and composites has expanded and become routine in dentistry, encompassing direct and indirect application, mainly due to the development of computer-aided design/computer-aided manufacturing (CAD/CAM) systems, which allow the fabrication of high-quality restorations with the various materials available, especially ceramics.[7–9] In an attempt to combine the mechanical properties of composite resin with the esthetic properties of conventional ceramics, resin-matrix ceramics have been introduced into the market for CAD/CAM restorations. They are formed by ceramic particles bonded to methacrylate-based polymeric networks. They have improved flexural strength and adhesion, and their elastic modulus is close to that of dentin.[1,5] Also, they are characterized by lower porosity, lower polymerization shrinkage and greater homogeneity of the material as compared to direct-application composites.[8] Research on stress distribution,[10,11] simulation models, as well as mathematical[12] or laboratory studies,[13] demonstrate that resin-based ceramics present reliability,[13] tend to concentrate the occlusal stress in the monolithic crown itself and along the cementation line,[11] not showing a significant difference in the intensity of strain on the tooth as compared to traditional ceramics,[10] and even in critical situations, e.g. in the case of bruxism, have a good expected clinical performance.[12]

Nevertheless, the resin matrix can degrade over time,[14–16] when exposed to environmental conditions, such as low pH and variations in the temperature. Due to the degradation of the organic matrix in such conditions, the sorption of some extrinsic pigments have been observed in composites,[14] potentially altering the esthetic color characteristics, as well as surface characteristics of the material, such as roughness and hardness.[6,8,15–18] The presence of water affects the structural integrity of the polymer, as the swelling of the polymer network is connected with increased distances between the network chains, which reduces the strength of the polymer.[5]

The stability of the esthetic characteristics of the material, as well as its performance, are affected by the conditions of the oral environment, with the frequent ingestion of certain types of foods and beverages causing pH fluctuations and staining.[19] Different types of solutions have been tested; however, studies investigating the color change of the CAD/CAM resin-matrix ceramics immersed in multiple common beverages at same time are scarce.[6,8,17,18] The molecular size and absorption properties of these materials are strongly related to change in their color when they are immersed in coffee, and influence their roughness when they are immersed in soft drinks like cola.[20] The various compounds present in red wine, such as alcohol and specific stains, may lead to color change in resin-matrix ceramics.[6] Tea is another beverage that can induce color change in these materials.[6,21]

The exposure of resin-matrix ceramics to the aforementioned beverages can potentially affect their optical and esthetic characteristics. Cola, coffee, energy drinks, fruit juice, and red wine are known to contain various colorants, acids and tannins, which can cause discoloration, and surface roughness and microhardness alterations.

Recent reports indicate that beverages such as cola, coffee, energy drinks, fruit juice, and red wine are widely consumed.[22,23] Considering that these beverages are globally prevalent, the present realistic approach adds novelty, as it directly addresses the impact of the beverages typically drunk by people, making the findings more relevant and relatable. The findings of this investigation can inform dental practitioners about the potential risk associated with certain beverages for patients with resin-matrix ceramic restorations. This knowledge can guide recommendations and post-treatment care instructions, ultimately promoting better patient outcomes.

In view of this, the purpose of the present study was to evaluate the effects of immersion of 2 resin-matrix ceramics in high-consumption beverages on their color stability, and surface roughness and microhardness. The null hypothesis of the study was that immersion in different beverages would not influence the stainability of the materials.

Material and methods

A polymer-infiltrated ceramic network (PICN) for CAD/CAM (VITA ENAMIC® (VE); VITA Zahnfabrik, Bad Säckingen, Germany) and a resin nanoceramic for CAD-CAM (Ambarino® High-Class (AH); CREAMED, Marburg, Germany) were evaluated in the present study (Table 1). The sample size calculation was performed with the use of the OpenEpi program (https://www.openepi.com), taking into account the mean difference at a confidence interval (*CI*) of 95% and power of 80%, based on a previous study that used PICN.[6] The color change (ΔE_{00}) values obtained for distilled water and cola were used, indicating a minimum required number of 2 samples per group. Considering the amount of material available for this research and the statistical robustness, 8 samples per group were obtained (n = 8).

Sample collection

Blocks for CAD/CAM from the 2 materials were sectioned under irrigation with a slow-speed diamond saw (IsoMet® 1000 Precision Saw; Buehler, Lake Bluff, USA). Forty-eight samples were obtained for each material with dimensions of 6 mm × 5 mm × 1 mm. The samples were polished under water cooling with 320-, 400-, 600-, and 1,200- grit sandpaper for 30 s on each grit. Then, they were immersed in a distilled water ultrasonic bath (Lavadora Ultrassônica Plus; Ecel, Ribeirão Preto, Brazil) for 10 min and gently dried with paper towels.

Staining procedures

The samples of each material were randomly divided into 6 groups (n = 8), according to the beverage used, as described in Table 2. The pH of the beverages was measured using a pH-indicator (PHS-3 BW; BEL Equipamentos Analíticos, Piracicaba, Brazil). Each sample was stored in a 1.5-millilter microtube. The beverages were distributed in the form in which they are commercialized, with the exception of coffee. Coffee was prepared by dissolving 5 g of soluble coffee in 50 mL of boiling distilled water in the proportion recommended by the manufacturer. Each microtube received 1.0 mL of a particular beverage. After dispensing the beverages, the microtubes were stored for 137 h at 37°C and 100% humidity.[24] The immersion period corresponded to 7.5 years of exposure, based on the following estimate: 3 coffees per day, multiplied by 1 min of exposure per cup, multiplied by 365 days per year, resulting in 1,095 min of exposure per year.[21] After the immersion period, the specimens were washed in distilled water and gently dried with paper towels.

The color, and surface roughness (Ra) and microhardness (KHN) readings were performed at 2 time points – initially and after the immersion period.

Table 1. Composition of the tested computer-aided design/computer-aided manufacturing (CAD/CAM) blocks according to the manufacturers

Resin-matrix ceramic	Code	Composition
VITA ENAMIC	VE	UDMA, TEGDMA (monomer); a feldspar ceramic enriched with aluminum oxide (filler)
Ambarino High-Class	AH	nanocharges, BDDMA, bis-GMA, UDMA (monomer); strontium boroaluminosilicate glass (filler)

UDMA – urethane dimethacrylate; TEGDMA – triethylene glycol dimethacrylate; BDDMA – 1,4-Butanediol dimethacrylate; bis-GMA – bisphenol A-glycidyl methacrylate.

Color analysis

The color data was recorded using the Delta Vista 450G spectrophotometer (Delta Color, São Leopoldo, Brazil), with an opening of 6 mm, directing light at an angle of 10°. The specimens were placed against a standard white background with a D65 illuminant, which represents the spectral distribution of daylight.[25] The coordinate data was collected with the use of the i7 Gold 1.0.3.5 software (Delta Color). The color difference (ΔE_{00}) is calculated

Table 2. Beverages used in the study and their pH values

Solution	pH	Brand
Distilled water	6.00	–
Cola	2.65	Coca-Cola® Sorocaba Refrescos, São Paulo, Brazil
Soluble coffee (5 g/50 mL)	4.88	Nescafé® Nestlé, Araras, Brazil
Energy drink	2.69	TNT Cervejaria Grupo Petrópolis, Teresópolis, Brazil
Orange juice	3.75	Life Sucos, Tabatinga, Brazil
Red wine	3.55	Pérgola Vinícola Campestre, Campestre da Serra, Brazil

based on the differences in coordinates L*, a* and b*. The L* values indicate luminosity, which ranges from black (0) to white (100). The a* values represent color change from red (+a*) to green (–a*) and b* values represent color change from yellow (+b*) to blue (–b*).[24] For this study, the following formula of CIEDE2000 (Commission internationale de l´éclairage (CIE) – the International Commission on Illumination) was used to interpret the coordinates (Equation 1)[20,26,27]:

$$\Delta E_{00}^{*} = \sqrt{\left(\frac{\Delta L'}{k_L S_L}\right) + \left(\frac{\Delta C'}{k_C S_C}\right) + \left(\frac{\Delta H'}{k_H S_H}\right) + R_T \frac{\Delta C'}{k_C S_C} \frac{\Delta H'}{k_H S_H}} \quad (1)$$

where ΔL', ΔC' and ΔH' are the differences in luminosity, chroma and hue between the final and initial evaluation, and R_T is the function that accounts for the interaction between chroma and hue in the blue spectrum. The S_L, S_C and S_H values refer to the functional balance for luminosity, chroma and hue, and k_L, k_C and k_H are parametric factors according to the visualization parameters, which are fixed at 1.[26,28]

Roughness analysis

The evaluation of surface roughness (Ra) was performed using a three-dimensional (3D) confocal laser microscope (LEXT OLS4000; Olympus, Tokyo, Japan), which captured an image with ×5 magnification. The Ra value represents the average difference in the mean height from the mean plane, providing stable results. Scratches, contamination or measurement noise are minimized by using this parameter. The entire digitized surface was analyzed with the use of the microscope software system (Olympus). The mean Ra values were calculated and expressed in micrometers. The variation between the final and initial Ra was calculated as: $\Delta Ra = Ra_f - Ra_i$.

Microhardness analysis

Surface microhardness (KHN) was measured with a microhardness tester (HMV-2; Shimadzu, Kyoto, Japan), under a load of 50 gf for 15 s and ×40 magnification. The surface of each sample was subjected to 3 Knoop indentations, 1 in the center and 2 in the opposite ends, 1 mm apart from the central region. The mean KHN value was the average of the 3 values obtained on each specimen. The variation between the final and initial KHN was calculated as: $\Delta KHN = KHN_f - KHN_i$.

Statistical analysis

The obtained data was tabulated, and statistical analysis was performed using the IBM SPSS Statistics for Windows software, v. 20.0 (IBM Corp., Armonk, USA). All data was normally distributed according to the Shapiro–Wilk and Kolmogorov–Smirnov normality tests ($p > 0.05$). The two-way analysis of variance (ANOVA) with the Bonferroni post-test was used for the statistical analysis of the color stability (ΔE_{00}, ΔL*, Δa*, and Δb*), and surface roughness (ΔRa) and microhardness (ΔKHN) data.

Results

The baseline values for the color coordinates of AH and VE were, respectively, 62.58 and 61.58 for L*, –0.158 and –0.213 for a*, and 6.748 and 9.651 for b*. Table 3 and Fig. 1 show the mean values of ΔE_{00}, ΔL*, Δa*, and Δb* after immersion in various beverages, with differences between the 2 materials within the same beverage group and between the beverage groups within the same material.

When comparing the materials with regard to the beverage group, there was a difference in ΔE_{00} for the groups of distilled water ($p = 0.043$), coffee ($p = 0.010$) and red

Table 3. Color change (ΔE_{00}), and the ΔL*, Δa* and Δb* coordinates after immersion in different beverages

Material	Beverage	ΔE_{00}	ΔL*	Δa*	Δb*
AH	distilled water	1.506 ±0.645^{Aac}	–1.315 ±0.733^{Aa}	–0.500 ±0.369^{Aa}	–0.226 ±0.660^{Aa}
	cola	2.470 ±0.862^{Aac}	–0.207 ±1.568^{Aa}	0.842 ±0.589^{Abce}	1.439 ±1.550^{Aab}
	coffee	4.459 ±2.071^{Ab}	–1.690 ±2.258^{Aa}	1.064 ±1.113^{Abe}	3.407 ±4.146^{Ab}
	energy drink	1.602 ±0.845^{Aac}	–0.562 ±1.171^{Aa}	0.005 ±0.554^{Aacd}	0.904 ±1.237^{Aa}
	orange juice	1.962 ±0.754^{Aac}	–0.624 ±0.915^{Aa}	0.419 ±0.358^{Abd}	1.769 ±1.285^{Aa}
	red wine	3.112 ±1.111^{Aabc}	–1.414 ±1.574^{Aa}	1.535 ±0.589^{Ae}	2.307 ±0.931^{Ab}
VE	distilled water	2.650 ±0.674^{Bac}	–2.129 ±0.984^{Aa}	–1.200 ±0.361^{Ba}	–0.670 ±0.690^{Aa}
	cola	3.556 ±1.288^{Aac}	–1.204 ±1.320^{Aac}	1.554 ±0.620^{Bb}	3.914 ±1.132^{Bbc}
	coffee	5.925 ±1.298^{Bb}	–4.361 ±1.341^{Bb}	2.359 ±0.573^{Bb}	5.157 ±1.097^{Bbc}
	energy drink	1.652 ±1.026^{Aa}	–1.171 ±1.403^{Aac}	–0.142 ±0.357^{Ac}	1.451 ±0.663^{Aa}
	orange juice	1.437 ±0.405^{Aa}	0.257 ±1.053^{Ac}	0.384 ±0.350^{Ac}	1.387 ±0.425^{Aa}
	red wine	7.280 ±1.392^{Bb}	–5.535 ±1.046^{Bb}	3.465 ±0.700^{Bd}	4.214 ±0.855^{Bc}

Data presented as mean ± standard deviation (*M ±SD*).
AH – Ambarino High-Class; VE – VITA ENAMIC. Different uppercase letters in superscript represent significant differences between the materials with regard to the same beverage, whereas different lowercase letters indicate significant differences between the beverages for each material.

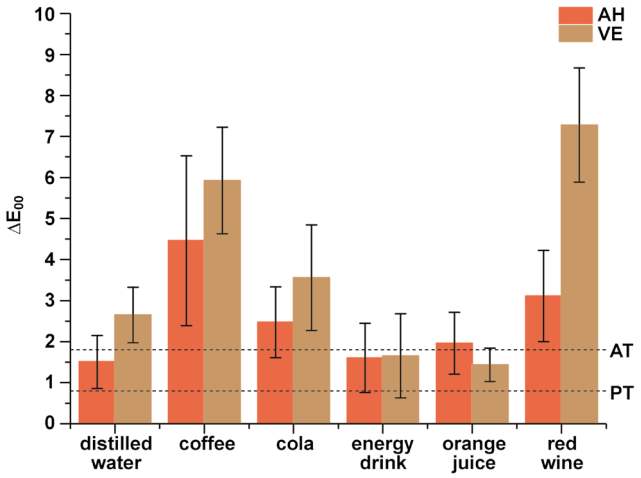

Fig. 1. Color change (ΔE_{00}) after immersion in different beverages in both material groups

Data presented as *M* ±*SD*.

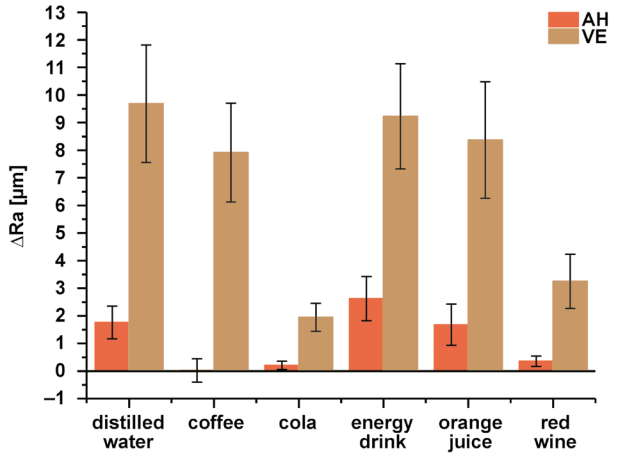

Fig. 2. Surface roughness change (ΔRa) after immersion in different beverages in both material groups

Data presented as *M* ±*SD*.

wine ($p < 0.001$). Exploring the coordinates, for the L* coordinate, there was a significant difference between the coffee ($p < 0.001$) and red wine ($p < 0.001$) groups. For the a* coordinate, there was a significant difference for the distilled water ($p = 0.019$), cola ($p = 0.017$), coffee ($p < 0.001$), and red wine ($p < 0.001$) groups, with greater variations presented by VE. For the b* coordinate, there was a significant difference for the cola ($p = 0.002$), coffee ($p = 0.025$) and red wine ($p = 0.015$) groups, with greater variations presented by VE.

Table 4 shows the mean values of ΔRa and ΔKHN after immersion in various beverages.

The ΔRa results show that AH differed from VE ($p < 0.05$), except when exposed to cola ($p = 0.168$) (Fig. 2). The type of beverage to which AH was exposed did not seem to influence the ΔRa results, while in the VE group, some differences could be detected between the beverages (immersion in cola and red wine presented a smaller roughness change as compared to other beverages tested): distilled water vs. cola ($p < 0.001$); distilled water vs. red wine ($p < 0.001$); coffee vs. cola ($p < 0.001$); coffee vs. red wine ($p = 0.005$); the energy drink vs. cola ($p < 0.001$); the energy drink vs. red wine ($p < 0.001$), orange juice vs. cola ($p < 0.001$); and orange juice vs. red wine ($p = 0.002$).

The ΔKHN results were different between AH and VE when the materials were immersed in the energy drink ($p = 0.018$) and orange juice ($p = 0.001$) (Fig. 3). Regardless

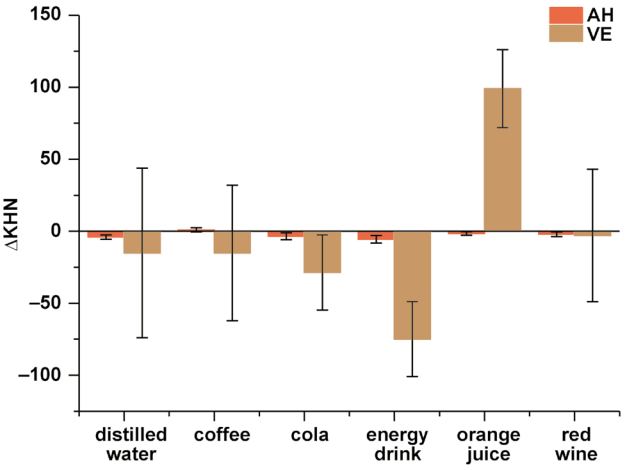

Fig. 3. Surface microhardness change (ΔKHN) after immersion in different beverages in both material groups

Data presented as *M* ±*SD*.

of the type of beverage in which AH was immersed, no significant difference was detected in ΔKHN. However, VE showed differences between the beverage groups (immersion in orange juice presented a greater microhardness change as compared to other beverages tested): distilled water vs. orange juice ($p = 0.002$); cola vs. orange juice ($p < 0.001$); coffee vs. orange juice ($p = 0.002$); the energy drink vs. orange juice ($p < 0.001$); and red wine vs. orange juice ($p = 0.010$).

Table 4. Changes in surface roughness (ΔRa) and microhardness (ΔKHN) after immersion in different beverages

Parameter	Material	Distilled water	Cola	Coffee	Energy drink	Orange juice	Red wine
ΔRa [μm]	AH	1.76 ±1.18^{Aa}	0.20 ±0.29^{Aa}	0.02 ±0.85^{Aa}	2.62 ±1.60^{Aa}	1.68 ±1.50^{Aa}	0.35 ±0.38^{Aa}
	VE	9.69 ±4.26^{Ba}	1.95 ±1.01^{Ab}	7.92 ±3.58^{Ba}	9.22 ±3.80^{Ba}	8.37 ±4.22^{Ba}	3.25 ±1.95^{Bb}
ΔKHN	AH	−4.05 ±2.83^{Aa}	−3.55 ±4.82^{Aa}	0.88 ±2.84^{Aa}	−5.56 ±5.16^{Aa}	−1.57 ±2.51^{Aa}	−2.06 ±3.19^{Aa}
	VE	−15.12 ±117.88^{Aa}	−28.63 ±52.11^{Aa}	−15.12 ±94.27^{Aa}	−74.95 ±51.88^{Ba}	99.00 ±54.38^{Bb}	−2.97 ±92.11^{Aa}

Data presented as *M* ±*SD*.
Different uppercase letters in superscript represent significant differences between the materials with regard to the same beverage, whereas different lowercase letters indicate significant differences between the beverages for each material.

Discussion

The predictability of the esthetics of restorations, even taking into account the possible stains present in the patient's food, is questioned in any restorative material. The present study evaluated the color, roughness and microhardness changes of PICN (VE) and a resin nanoceramic (AH) after immersion in different beverages that have a recognized staining effect on restorative materials. The null hypothesis of this study was rejected, since the beverages had a significant effect on color, roughness and microhardness change.

Paravina et al. calculated the color difference thresholds related to perceptibility (PT: ΔE_{00} = 0.8) and acceptability (AT: ΔE_{00} = 1.8) for ceramic materials under simulated clinical settings,[29] with better correlation with the human perception of color differences.[30,31] Using these reference values, ΔE_{00} for the resin-matrix ceramics tested in the current study exceeded the PT value; however, the resin nanoceramic AH presented acceptable values for the samples immersed in distilled water and the energy drink, and the PICN VE presented acceptable values for the energy drink and orange juice groups. The other immersion solutions produced ΔE_{00} values above the limits of acceptability after simulating 7.5 years of exposure.

The type of matrix influences the color stability of the resin-matrix ceramic, as it affects water sorption.[1,2] The fraction of polymers in the resin matrix is not directly correlated to ΔE_{00}, which can be better explained by the characteristics of the monomers present in the resin matrix composition.[3] Materials with greater hydrophilicity are susceptible to staining.[2] VE is composed of hydrophobic urethane dimetacrylate (UDMA) and triethylene glycol dimethacrylate (TEGMA). The TEGMA present in the VE composition possibly causes an increase in water sorption by the material, which brings hydrophilic dyes to the resin matrix.[1,17,32] Dimethacrylates form cross-linked networks with the unreacted monomers, acting as plasticizers along the polymeric network, leaving the structure open and facilitating sorption.[1,5] In addition, the polymeric matrix, when degraded, exposes the ceramic network, creating a surface more prone to the accumulation of stains.[6]

Another monomer, bisphenol A-glycidyl methacrylate (bis-GMA) present in the AH composition, can also trigger the same pigmentation mechanism in the polymeric matrix,[3] exhibiting more hydrophilicity and water sorption than other monomers found in the composition of the resin-matrix ceramics tested in this study.[3,6,17,33,34] If a polymer can absorb water, it can also absorb water-soluble pigments.[8] However, the combination of monomers and the amounts used possibly affected the color stability of the resin-matrix ceramics and their ability to resist staining, since AH showed lower ΔE_{00} values in almost all the tested solutions as compared to VE. According to the manufacturers, AH has 30% by weight of the resin matrix, while VE has 14%. Even with a greater amount of polymers in its composition, AH showed lower values of ΔE_{00}, which suggests that the combination and amounts of the monomers used for the AH resin matrix resulted in less pronounced sorption and embedding of water-soluble pigments as compared to the VE resin matrix.

In previous studies evaluating the color stability of a material, beverages such as coffee and red wine were usually chosen as the staining solutions.[9,14,17,35] In the present study, in addition to coffee and red wine, solutions such as cola, the energy drink and orange juice were included because of their large consumption, and distilled water was used for comparison.[18] Due to phenolic components, such as tannins and anthocyanins, red wine has an effect on the color of restorations,[3,6] potentiated by ethanol and low pH, which cause the degradation of the resin matrix, its penetration and the absorption of colorants.[6] The acidic and alcoholic properties of red wine generate an increase in Ra and alterations in the surface topography.[14] The bipolar molecule of ethanol can facilitate the solubilization of hydrophilic or hydrophobic components, thus softening and decomposing the resin matrix.[3] In this study, red wine caused ΔE_{00} above the AT value (ΔE_{00} > 1.8), mainly in the VE group. When ΔRa was evaluated after exposure to red wine, VE showed a greater increase than AH. This can be explained by the fact that VE has monomers in its resin matrix composition that are more hydrophilic than the monomers present in the AH resin matrix. With regard to KHN, the exposure of AH and VE to red wine did not provide statistically different results, decreasing the parameter in both groups.

Methacrylate-based materials are more susceptible to staining when exposed to coffee because of the absorption of dyes, which probably occurs due to the affinity of the polymer network to the coffee yellow stain molecules of low polarity.[1,2,8] The results of the b* coordinate analysis show that the coffee group, when compared to distilled water, demonstrated an increase in the saturation of the yellow chroma, and AH had better resistance to staining. Coffee is naturally acidic, containing about 22 types of acid, including citric, oxalic and malic acids. When consumed, it causes chemical erosion on the surface of the resin-matrix ceramic.[6] VE is composed of a ceramic network infiltrated by a polymeric matrix, which is exposed by the hydrolysis of the methacrylate ester bonds in the resin matrix[36] when submitted to acid erosion, leading to a rougher surface than in the case of AH; the latter material presented a lower ΔRa value and better resistance to staining after exposure to coffee in this study. On the other hand, water-soluble anthocyanins responsible for the red color of wine are more polar pigments than the yellow pigments of coffee, penetrating the polymeric network deeper.[1] Nevertheless, red wine caused greater alterations relative to the a* coordinate in the resin-matrix ceramic VE.

Rough surfaces affect the reflection of light on the material, generating a pattern of diffused and irregular

reflection, and consequently impacting the color and appearance of the restoration.[36] Another property that influences the esthetics of a restoration is translucency. It depends on the thickness and superficial roughness of the material used.[3,6] In this study, immersion in the solutions did not affect the Ra of AH, while VE showed altered values for the coffee, energy drink, orange juice, and red wine groups. VE is a material that has a polymeric matrix associated with a ceramic network based on the feldspar ceramic particles. As the matrix degrades due to low pH and hydrolysis, ceramic particles can be exposed or detached, increasing Ra.[6] Greater Ra favors the deposition of pigments on the material surface, decreasing brightness, and consequently increasing ΔE_{00}.[6,14] The increased Ra of VE may also be related to the ΔE_{00} found.

The highest sorption values are found in the materials immersed in solutions with pH between 4 and 6.[1] Sorption influences the degradation[14] and staining of the polymeric matrix.[1,3] In the evaluated beverages, only coffee has pH in this range, which can explain the ΔE_{00} results for the AH and VE samples immersed in coffee. Alnasser et al. demonstrated in a previous study the effect of pH on a resin-matrix ceramic, where a significant increase in Ra was observed after immersion in an acidic solution (pH 2.0).[36] In the present study, cola, coffee, the energy drink, orange juice, and red wine with their acidic pH showed increased Ra, but the solutions apparently had the most powerful effect on the Ra of VE, with significant differences noted for all experimental groups, except cola, when compared to AH, suggesting that the VE resin matrix is more susceptible to the changes caused by exposure to the tested solutions than AH. The control group (distilled water) in the VE samples also showed an increase in Ra, which could be related to the hydrophilicity of the polymeric matrix. However, further analysis would be necessary to state this with absolute certainty.

The literature shows decreased microhardness after thermocycling,[7,15,37] and exposure to acidic media[16,38] and high-consumption beverages with low pH.[1,39] The results of this study show that cola, coffee, the energy drink, and red wine generally decreased KHN in both material groups, highlighting the decrease found in VE when immersed in the energy drink. However, orange juice showed decreased KHN in AH and increased KHN in VE. According to the information provided by the manufacturer, VE is a material with particles of feldspatic ceramics in its composition, besides SiO_2, NaO_2 and K_2O in the matrix mainly composed of UDMA.[1] When the UDMA matrix degrades, feldspatic ceramic particles can be exposed. The Knoop diamond penetrator used in the hardness test has a long, diagonal shape, which in view of the exposure of the particles due to the degradation of the polymeric matrix, could have influenced the results obtained in this study by affecting the complete penetration of the indentation into the surface of VE.

The Ra of the material is important in terms of the patient's comfort and reducing the formation of surface fractures, which is often achieved by applying a glaze layer.[40] The application of a glaze layer also favors the maintainance of color stability, even in the conventional glass ceramics when immersed in the staining solutions.[24] In resin-matrix ceramics, stain covers give a better effect with regard to appearance and gloss, which are retained by a glaze layer.[41,42] The finishing systems are based on the application of photoactivatable stain covers and glazing, from the acid-etching or sandblasting of the surface to its silanization.[41,43] As an alternative to glazing, the creation of a smoother surface can also be achieved by polishing.[32] However, the polishing and finishing systems chosen can affect the absorption of water by resin-matrix materials,[44] which might affect color stability by the absorption of water-soluble pigments. Still, it appears to be a viable alternative to extrinsic stain removal.[5] In this study, no surface finishing system was applied to the samples of any material. Thus, the samples might have been more susceptible to color and surface roughness changes. An issue to be highlighted is the possibility of the light-curing of the stain or performing effective polishing on the surface of a resin-ased ceramic in the chairside method.

The limitations of this study can be attributed to the fact that in the oral environment, the materials, while being exposed to different pigments, are also aided by the salivary flow, which dilutes the concentration of these pigments and has the buffer effect, which was not simulated in the experimental model used. Toothbrushing and restoration surfaces can prevent surface staining, but toothbrushing was not simulated in this study, probably affecting the results.[34]

There is still scope for future studies using aging methods, and the specific finishing and glazing systems. These methods can be combined with immersion in high-consumption beverages and toothbrushing to better understand the color change behavior of these materials. Specific studies for the monomers used in the resin-based matrix and their properties when combined could also be useful in the improvement and development of these materials. The use of scanning electron microscopy (SEM) could have solved some questions that occurred during the realization of this study. Images from this type of microscopy could provide a better understanding of the bonding of the resin matrix to the filler particles in the materials tested and the particle size of each material. In addition to a surface analysis that could point to different surface effects for each beverage, future studies using SEM must be conducted.

Conclusions

Based on the results, it can be concluded that the consumption of the beverages tested, given their low pH and

the pigments they contain, may significantly affect the esthetics and surface characteristics of the resin-matrix ceramics tested, including their color, roughness and microhardness. Minimizing exposure to these beverages, along with good cleaning conduct during toothbrushing, should be considered whenever these materials are chosen for dental rehabilitation to minimize adverse effects and maintain the restoration with no need for substitutions over time.

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.

ORCID iDs

Ebele Adaobi Silva https://orcid.org/0000-0002-4204-1121
Anselmo Agostinho Simionato https://orcid.org/0000-0002-6913-0001
Olívia Breda Moss https://orcid.org/0000-0002-1425-2247
Adriana Cláudia Lapria Faria https://orcid.org/0000-0001-6353-1480
Renata Cristina Silveira Rodrigues https://orcid.org/0000-0003-4140-4143
Ricardo Faria Ribeiro https://orcid.org/0000-0003-4211-0542

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Original paper

Global co-occurrence of bruxism and temporomandibular disorders: A meta-regression analysis

Grzegorz Zieliński[1,A–F], Beata Pająk-Zielińska[2,B,D,F], Agnieszka Pająk[3,B,D,F], Marcin Wójcicki[4,E,F], Monika Litko-Rola[4,E,F], Michał Ginszt[5,E,F]

[1] Department of Sports Medicine, Medical University of Lublin, Poland
[2] Interdisciplinary Scientific Group of Sports Medicine, Department of Sports Medicine, Medical University of Lublin, Poland
[3] Clinic of Anaesthesiology and Paediatric Intensive Care, Medical University of Lublin, Poland
[4] Independent Unit of Functional Masticatory Disorders, Medical University of Lublin, Poland
[5] Department of Rehabilitation and Physiotherapy, Medical University of Lublin, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):309–321

Address for correspondence
Grzegorz Zieliński
E-mail: grzegorz.zielinski@umlub.pl

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on January 10, 2025
Reviewed on February 9, 2025
Accepted on February 12, 2025

Published online on March 18, 2025

Abstract

Background. Bruxism and temporomandibular disorders (TMD) are closely related, yet the relationship between bruxism and TMD remains one of the most debated topics in the literature.

Objectives. The aim of the study was to estimate the overall proportions of the co-occurrence of bruxism and TMD, and the prevalence of TMD in individuals with bruxism by continent. Additionally, factors that have an influence on these proportions, including geographical region, sex and other demographic variables, were analyzed.

Material and methods. A synthesis of data from 6 meta-analyses and systematic reviews published up to October 2024 was conducted. The data was extracted from 30 studies that analyzed 31 populations, with a total of 37,680 participants, of whom 5,117 were diagnosed with both bruxism and TMD. The analyses were conducted using the R statistical language.

Results. The global co-occurrence of bruxism and TMD was 17%, with significant differences observed between continents. In North America, the co-occurrence of these 2 conditions was 70%, followed by 24% in South America, 14% in Europe and 9% in Asia. The analysis revealed that the sex of the participants was a significant factor, as higher proportions of female participants in a study sample increased the likelihood of the co-occurrence of TMD and bruxism. The mean prevalence of TMD among patients with bruxism was 63.5%, with the highest rate observed in North America (98.3%) and the lowest in Asia (53.9%).

Conclusions. The meta-analysis underscores the high prevalence of TMD in individuals with bruxism, highlighting significant geographical variations in the co-occurrence of these conditions. A 1% increase in the proportion of female participants in a study group was associated with a 4.4% rise in the probability of the co-occurrence of TMD and bruxism. These findings suggest that temporal factors and the average age of participants did not significantly contribute to observed variability across studies. The results underscore the importance of geographical and demographic factors in understanding the interplay between bruxism and TMD.

Keywords: TMD, bruxism, temporomandibular disorders, association, connection

Cite as
Zieliński G, Pająk-Zielińska B, Pająk A, Wójcicki M, Litko-Rola M, Ginszt M. Global co-occurrence of bruxism and temporomandibular disorders: A meta-regression analysis. *Dent Med Probl.* 2025;62(2):309–321. doi:10.17219/dmp/201376

DOI
10.17219/dmp/201376

Highlights

- The global prevalence of bruxism and TMD co-occurence is 17%, with regional variations: 70% in North America; 24% in South America; 14% in Europe; and 9% in Asia.
- A 1% increase in the female proportion in a study sample raises the probability of co-occurrence by 4.4%.
- The overall prevalence of TMD among individuals with bruxism is 63.5%, with North America showing the highest prevalence at 98.3%.
- Geographical and demographic factors influence the co-occurrence of bruxism and TMD, highlighting the need for further research, particularly in North America.

Introduction

Temporomandibular disorders (TMD) are a group of conditions characterized by dysfunction and pain in the temporomandibular joint (TMJ), masticatory muscles and surrounding tissues. Symptoms of TMD may include facial pain, difficulty opening the mouth, joint clicking or popping, and limited jaw mobility.[1]

According to the 2018 consensus,[2] bruxism is defined as the activity of the masticatory muscles, which involves either grinding or clenching the teeth and/or bracing or pushing the jaw. This condition is categorized into 2 types: sleep bruxism (SB); and awake bruxism (AB). Sleep bruxism manifests during sleep and can be either rhythmic or irregular, whereas awake bruxism occurs while awake and involves repetitive or prolonged tooth contact and/or jaw movement. Bruxism is not considered a disorder in healthy individuals but rather a behavior that could serve as a risk factor or offer protection for certain medical conditions.[2]

Bruxism and TMD share a multifactorial etiology encompassing biological (e.g., genetic and anatomical), psychological (e.g., stress, emotional disorders) and environmental aspects (e.g., lifestyle, habits). The co-occurrence of bruxism and TMD underscores the complex interaction between these factors, which complicates the determination of their precise etiology and necessitates a comprehensive diagnostic and therapeutic approach.[3–6]

Manfredini and Lobbezoo emphasize that the relationship between bruxism and TMD remains among the most debated topics in dental literature, mainly due to uncertainties surrounding etiological and diagnostic aspects of both conditions.[7]

Over the years, several systematic reviews have analyzed the association between bruxism and TMD. A systematic review conducted by Manfredini and Lobbezoo identified a positive relationship between bruxism and TMD pain, based on studies that used self-diagnosis or clinical diagnosis of bruxism.[7] However, these studies were subject to potential biases and confounding factors, while more quantitative and specific research methods have demonstrated a much weaker association. The authors observed that experimental jaw clenching did not reflect clinical TMD pain.[7] A systematic review by Jiménez-Silva et al. suggested a possible association between sleep bruxism and myofascial pain, arthralgia, and joint pathologies.[8] A meta-analysis by Mortazavi et al. found a positive relationship between bruxism and TMD, with the presence of bruxism increasing the likelihood of future TMD development.[9] In contrast to the abovementioned associations, polysomnographic studies have not observed a connection between SB and TMD.[10–12] The most recent study demonstrated that SB is more frequently associated with TMJ pain and functional jaw limitations than AB.[13] This observation highlights the complexity of the issue and the need for further research.[14]

In 2024, 2 meta-analyses examined the prevalence of TMD[3] and bruxism[4] across continents. These studies indicated that geographical factors may influence the prevalence of these conditions. The global prevalence of TMD was estimated at 34%,[3] while the global prevalence of bruxism (both SB and AB) was estimated at 22.22%.[4] When analyzed separately, the prevalence of SB was estimated at 21%, and the prevalence of AB at 23%.[4] The continental analysis revealed a high co-occurrence of bruxism and TMD in the Americas (Fig. 1). However, these findings do not allow for a definitive determination of whether bruxism and TMD co-occur due to interdependence.

A review of the available literature did not yield a conclusive answer regarding the inherent association between bruxism and TMD. Consequently, a meta-regression analysis was conducted based on the synthesized data from previously published meta-analyses and systematic reviews.

The primary objectives of the study were to estimate the pooled prevalence of (1) bruxism (both SB and AB) and TMD co-occurrence and (2) TMD prevalence among individuals with bruxism across continents. The influence of continents on these proportions was assessed using the meta-regression, with continents serving as categorical moderators. Based on previously published research analyzing the occurrence depending on the continent,[3,4] a hypothesis was formulated that both phenomena would co-occur, particularly with regard to North and South America.

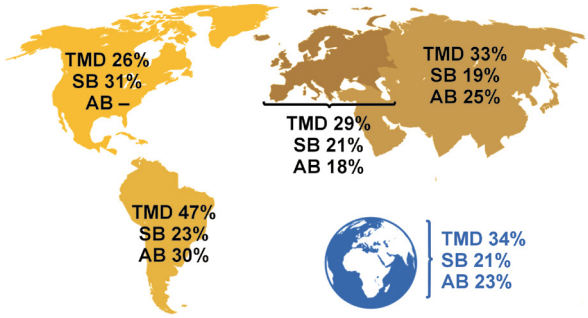

Fig. 1. Results of epidemiological studies on temporomandibular disorders (TMD) and bruxism by continent[1,2]

SB – sleep bruxism; AB – awake bruxism.

Material and methods

The project was registered in the Open Science Framework (OSF) database (https://osf.io/2afqr). The research was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) 2020 checklist.[15] The project commenced on October 29, 2024. The following terms were used to search PubMed®, Web of Science and Scopus databases: "Bruxism"; "Temporomandibular Disorders"; "TMD"; and "Systematic Reviews".[3,4,16] The search was conducted for articles published before October 29, 2024. Only systematic reviews and meta-analyses investigating the co-occurrence of bruxism and TMD were included in the study.

In the first phase of the study, the search was performed independently by 2 authors (BPZ and AP), and any discrepancies were resolved by the third author (GZ). Using the specified keywords, a total of 239 records were retrieved from the 3 selected databases. Titles were initially reviewed, resulting in the exclusion of 222 records. Subsequently, abstracts were analyzed and duplicates were removed. Six full-text articles were selected for further analysis. The following systematic reviews and meta-analyses were accepted for data extraction: Manfredini and Lobbezoo[7]; Jiménez-Silva et al.[8]; Mortazavi et al.[9]; de Oliveira Reis et al.[17]; Achmad et al.[18]; and Al-Jewair et al.[19]

The second phase of the study involved importing data from the included articles. Data analysis and import were conducted independently by 2 authors (BPZ and AP) under the supervision of the third author (GZ). In the event of any disagreement between the 2 authors, the supervisor was to make the final decision. However, this situation did not occur, as there was unanimous agreement between the 2 authors. Based on the 6 studies, data from 30 individual studies examining 31 populations were imported for the analysis of the co-occurrence of bruxism and TMD (Fig. 2).[20–49] The 31 analyzed populations included a total of 37,680 participants, 5,117 of whom were diagnosed with both bruxism and TMD. Detailed information about the studies are provided in the supplementary materials (Table A.1; https://osf.io/c38eg). The flow diagram was adapted from the PRISMA guidelines (Fig. 2).[15]

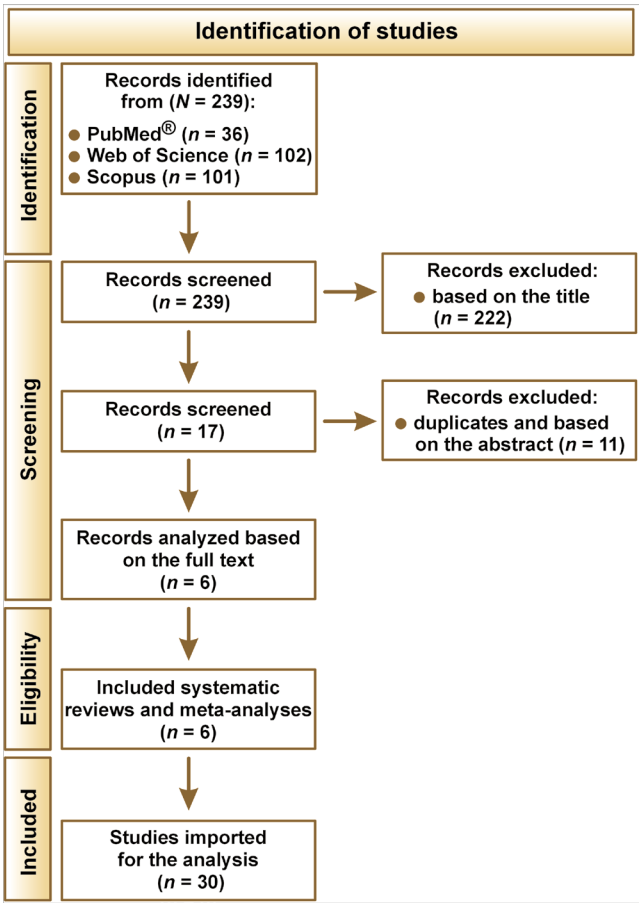

Fig. 2. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram of the study

Statistical analysis

A meta-analysis was conducted to estimate the pooled prevalence of events across studies. The analysis was performed using the generalized linear mixed model (GLMM) framework, which allows for the incorporation of both within-study and between-study variability. The logit transformation of proportions was applied as the effect size measure, as is recommended for meta-analyses involving proportions to stabilize variance and ensure appropriate weighting of studies.[50] The inverse variance method was used for weighting study estimates.

The confidence intervals for the pooled estimates were calculated using the normal approximation interval based on the summary measure method, which provides robust interval estimation for proportions.[51]

The back-transformation of pooled logit proportions to the original scale was applied to enhance the interpretability of the findings. Statistical analyses were performed without assuming a common heterogeneity estimate across subgroups (τ^2 not constrained to be common), allowing for subgroup-specific variability. Additionally, a random-effects model was employed, as it is more appropriate when clinical or methodological diversity is expected among included studies.[52] It accounts for

heterogeneity by assuming that the true effect sizes may vary across studies rather than being fixed.

Heterogeneity was quantified using several metrics. The between-study variance (τ^2) was estimated using the maximum likelihood (ML) method, which provides a likelihood-based estimate of heterogeneity and is particularly useful for meta-analyses with substantial variability.[53] The τ^2 value was then used to compute τ, representing the standard deviation of the true effect sizes. The I^2 statistic, which quantifies the percentage of total variation in effect estimates attributable to heterogeneity rather than sampling error, was calculated as described by Higgins and Thompson.[54] Additionally, the H-statistic, an alternative to I^2 for assessing heterogeneity, was computed to offer further insights into the degree of variability across studies.

Heterogeneity was formally assessed using both the Wald test and the likelihood ratio test (LRT). The Wald test evaluates heterogeneity by comparing the observed variance to the expected variance under the homogeneity assumption.[51] The likelihood ratio test compares the likelihood of the data under the random-effects model to the likelihood under a fixed-effects model, providing a more robust assessment of heterogeneity, particularly in datasets with high variability.[55]

Subgroup differences were evaluated using a random-effects model for subgroup analysis, which allows for the estimation of between-group variability while accounting for within-group heterogeneity. The assessment of subgroup differences was conducted using the Q-statistic, as described by Borenstein et al.[56] A forest plot was used to visually depict the results of the GLMM framework.

Publication bias was assessed using a combination of visual and statistical methods. Visual inspection of the funnel plot was used to identify patterns of asymmetry that might suggest potential bias.[57] The statistical tests for funnel plot asymmetry included Begg's rank correlation test and Peters' linear regression test. Begg's test evaluates the correlation between study effect sizes and their variances, relying on a rank-based approach to detect asymmetry.[58] Peters' test assesses the relationship between study effect sizes and their precision, using a linear regression framework that is particularly sensitive to detecting asymmetry in meta-analyses of proportions.[59]

The influence analysis was conducted to identify studies with a disproportionate impact on the meta-analytic results. The analysis employed a range of statistical methods, including standardized residuals, difference in fits (DFFITS), Cook's distance, covariance ratio, leverage ("hat"), and leave-one-out diagnostics. Standardized residuals identified potential outliers, while DFFITS and Cook's distance detected influential studies that affected predicted values and overall model fit.[51,60] The covariance ratio and leverage assessed the impact of individual studies on the precision of the model. The leave-one-out analysis evaluated changes in heterogeneity (τ^2, Q) and pooled estimates after excluding each study. The use of diagnostic plots facilitated the visualization of these metrics, enabling the identification of influential studies.

In cases where the test for subgroup differences yielded significant results, a meta-regression was conducted to examine the potential influence of moderators on effect sizes and to identify which subgroups exhibited significant differences. The analysis was performed using a mixed-effects model, incorporating both fixed effects for moderators and a random effect to account for between-study variance.[53] The restricted maximum likelihood (REML) method was used to estimate the residual heterogeneity (τ^2), providing a robust estimate of variability not explained by the included moderators.[51] The significance of individual predictors was assessed using Wald-type z-tests, while the overall significance of all moderators was evaluated using the omnibus test of moderators (Q_M), which compares the model with moderators to a null model without moderators. The statistical significance of moderators was determined at a predefined alpha level ($\alpha = 0.05$).

The papers were initially exported to Zotero, v. 6.0.36 (Corporation for Digital Scholarship, Vienna, USA). Subsequently, the data from the papers was exported to Microsoft Excel (Microsoft Corp., Redmond, USA). The analyses were conducted using the R Statistical language, v. 4.3.3 (https://cran.r-project.org/src/base/R-4) on Windows 11 Pro 64 bit (build 22631; Microsoft Corp.), using the meta (v. 7.0.0),[61] dmetar (v. 0.1.0),[62] report (v. 0.5.8),[63] ggplot2 (v. 3.5.0),[64] dplyr (v. 1.1.4),[65] and psych (v. 2.4.6.26) packages.[66]

Results

A total of 31 studies comprising 37,680 individuals, among whom 5,117 reported the co-occurrence of bruxism and TMD, were included in the analysis. The random-effects model yielded an overall pooled prevalence of 17.1% (95% confidence interval (*CI*): 11.4–24.9). However, the heterogeneity among the included studies was found to be extremely high (I^2 = 99.4%, 95% *CI*: 99.3–99.5), indicating that nearly all observed variability in the effect sizes was due to differences between the studies rather than a random error. This finding was further corroborated by high residual heterogeneity ($\tau^2 = 1.76$) and the Q statistic (Q = 4983.69, $p < 0.001$). Additionally, the LRT confirmed the presence of significant heterogeneity (LRT = 5876.73, $p < 0.001$).

Subgroup analysis by continent revealed substantial differences in the pooled proportions. Specifically, North America exhibited the highest pooled prevalence of bruxism and TMD co-occurrence at 69.8% (95% *CI*: 61.0–77.4). This finding was accompanied by moderate heterogeneity (I^2 = 79%, $\tau^2 = 0.0357$), based on only 2 studies. South America exhibited a pooled prevalence

of 24.1% (95% *CI*: 15.1–36.3), characterized by higher heterogeneity (I^2 = 96%, τ^2 = 0.6406). Asia demonstrated the lowest pooled prevalence at 9.4% (95% *CI*: 3.4–23.3), with heterogeneity remaining extremely high (I^2 = 99%, τ^2 = 1.7883). Europe, with the largest number of included studies (n = 15), exhibited a pooled prevalence of 13.7% (95% *CI*: 8.0–22.3), accompanied by very high heterogeneity (I^2 = 100%, τ^2 = 1.3529) (Fig. 3).

The test for subgroup differences revealed statistically significant variation in pooled prevalence across continents (Q = 79.09, $p < 0.01$). This finding suggests that geographical location may play an important role in the observed differences in the co-occurrence of bruxism and TMD (Fig. 3).

The extreme heterogeneity observed across studies and subgroups underscores the necessity for the establishment of standardized diagnostic criteria and methodologies in future research in order to better understand the global burden of bruxism and TMD co-occurrence.

The influence analysis reveals that while certain studies have a measurable impact on the results of the meta-analysis, no single study exerts a significant influence (Figure A.1, supplementary materials; https://osf.io/c38eg). High heterogeneity remains a concern, and specific studies contributing to this variability should be further reviewed. The overall pooled estimate demonstrates stability, suggesting that the observed variability is attributable to broader differences across the included studies rather than isolated outliers.

The results of the pooled proportions are displayed in Fig. 3 as forest plots, with each individual study listed on the left, grouped by continent, and represented by a square and a horizontal line. The square denotes the study's effect size (proportion), and its dimensions reflect the weight of the study in the meta-analysis, with larger squares signifying a greater influence. The horizontal line extending from each square represents the 95% *CI* of the effect size. A longer line indicates greater uncertainty, while a shorter line reflects more precision in the estimate. The co-occurrence of bruxism and TMD by continent subgroups is further visualized in Fig. 4.

The vertical dashed line in the plot signifies the null effect, which serves as a reference point for comparison. Studies with squares and *CI*s entirely to the right of the line suggest positive proportions, while those positioned to the left indicate negative or lower proportions. At the bottom of each subgroup, a diamond is displayed, providing a summary of the pooled estimate for that subgroup. The center of the diamond denotes the pooled effect size, while the width reflects the 95% *CI* of the subgroup. Narrower diamonds indicate more precise pooled estimates. Similarly, the diamond situated at the very bottom of the plot signifies the overall pooled effect size across all studies, providing a summary of the total effect.

As illustrated in Fig. 3, the 95% *CI* values overlapped across all continents, with the exception of North America. This finding indicates that there are no statistically significant differences between the proportions of individuals with bruxism and TMD reported for Asia, Europe and South America. However, to formally evaluate the significance of differences in proportions between continents, a meta-regression analysis was performed, with North America designated as the reference category.

To account for potential confounding effects, the meta-regression model was adjusted for a key sociodemographic factor: the proportion of females in the study sample. This value varied widely across studies, ranging from 15.5% to 85.6%, with a median of 64% (*IQR*: 51.24–79.22). The inclusion of this adjustment in the analysis was intended to provide a more accurate assessment of the geographical differences in the co-occurrence of bruxism and TMD, while accounting for the variability in sex distribution across studies.

The meta-regression analysis demonstrated significant differences in the co-occurrence of bruxism and TMD across continents. Specifically, North America exhibited the highest prevalence of bruxism and TMD compared to Asia and Europe. South America did not differ significantly from North America. The proportion of females in the study sample was also found to be a significant moderator, with higher values associated with increased prevalence of the co-occurrence of both conditions (Table 1). These findings highlight the importance of geographical and demographic factors in the understanding of the co-occurrence of bruxism and TMD.

Furthermore, there were no significant differences in the co-occurrence of the 2 conditions across Asia, Europe and South America.

Additional meta-regression models were conducted to evaluate the potential impact of other predictors, such as the year of the survey and the mean age of the studied individuals, on the co-occurrence of bruxism and TMD. The results indicated that neither the year of the survey (p = 0.508) nor the mean age of the study participants (p = 0.362) had a statistically significant effect on the co-occurrence of the 2 conditions. These findings imply that temporal factors and the average age of participants did not meaningfully contribute to the variability in the observed proportions across studies.

The funnel plot presented in Fig. 5 visually depicts the relationship between the logit-transformed proportion (effect size) and the standard error for each study included in the meta-analysis. Each circle corresponds to an individual study, with its position on the x-axis indicating the effect size and its position on the y-axis showing the level of precision (inversely proportional to the standard error). Studies with larger standard errors (smaller sample sizes) are positioned near the bottom of the plot. The dashed lines represent pseudo-confidence limits, which indicate the expected distribution of studies in the absence of bias.

The funnel plot is generally symmetrical, indicating that publication bias may not constitute a significant concern

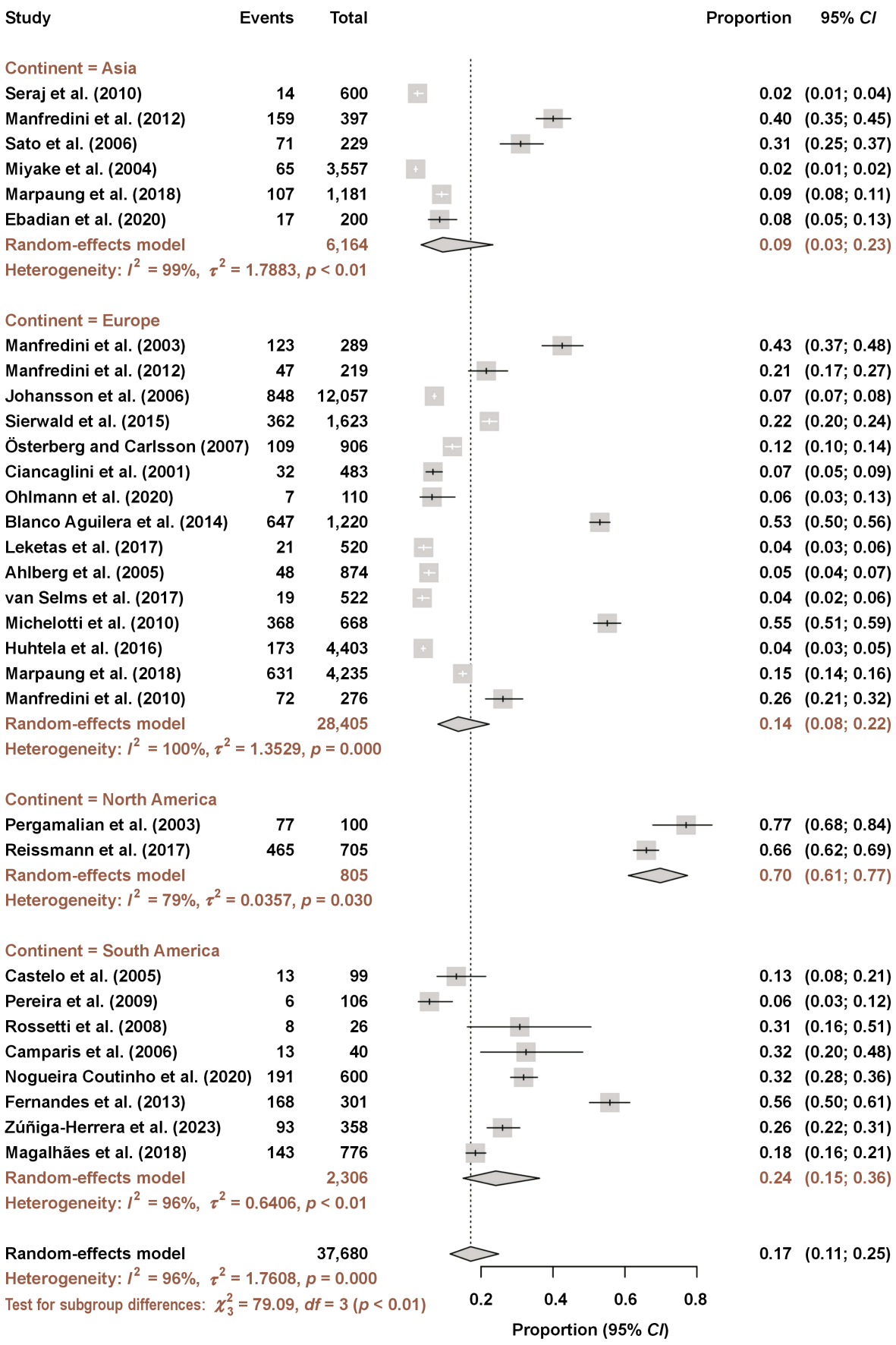

Fig. 3. Forest plot for the overall proportion of co-occurrence of bruxism and temporomandibular disorders (TMD) among subgroups by continent
CI – confidence interval; *df* – degrees of freedom.

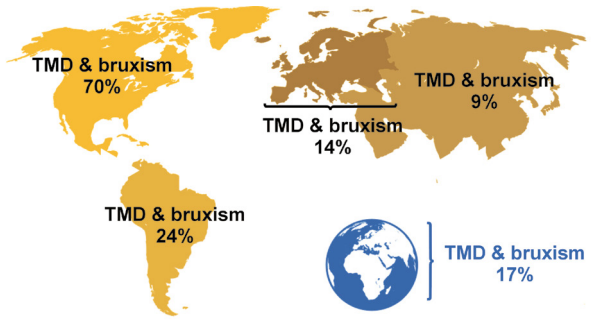

Fig. 4. Graphical representation of the results of the analyzed studies on the co-occurrence of temporomandibular disorders (TMD) and bruxism

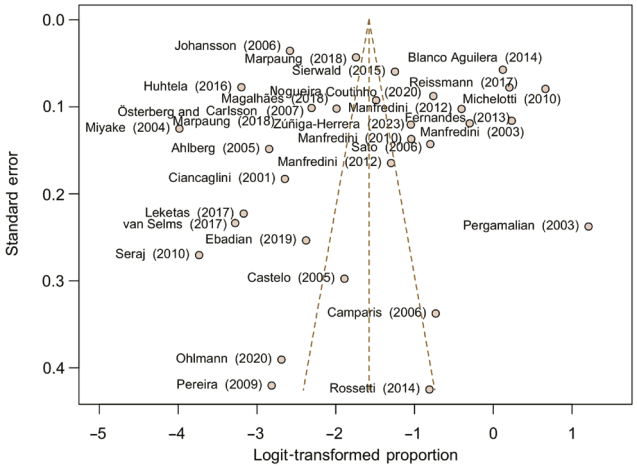

Fig. 5. Funnel plot assessing publication bias in the meta-analysis on the co-occurrence of bruxism and temporomandibular disorders (TMD) among adult patients

within the context of this meta-analysis. The majority of studies are situated within the pseudo-confidence limits, particularly those with smaller standard errors (larger sample sizes). However, a few studies with larger standard errors and extreme effect sizes, such as those on the far left (e.g., Ohlmann et al.[35] and Pereira et al.[21]), fall outside the pseudo-confidence limits. These studies imply the presence of heterogeneity or methodological discrepancies rather than systematic publication bias.

The assessment of funnel plot asymmetry was performed using 2 statistical methods: Begg's rank correlation test; and Peters' linear regression test. Peters' test, which evaluates asymmetry based on the inverse of the total sample size, yielded a bias estimate of 137.68 (standard error (*SE*) = 88.76), with a test statistic of t (29) = 1.55 (p = 0.132). This finding suggests an absence of statistically significant evidence supporting small-study effects or publication bias. Similarly, Begg's rank correlation test, which examines the association between effect sizes and their variances, demonstrated a bias estimate of −55.00 (*SE* = 58.84, z = −0.93, p = 0.349), providing no significant evidence for funnel plot asymmetry. The results of both tests suggest that the outcomes of the meta-analysis were not influenced by publication bias or the effects of small studies.

Meta-analysis of the pooled prevalence of TMD among subjects diagnosed with bruxism

A meta-analysis was conducted to estimate the pooled prevalence of TMD among adult patients with bruxism, stratified by continent. The analysis included 29 studies (after excluding the study by Marpaung et al.[47] as an influential study[51]), comprising a total of 8,462 participants with bruxism, of whom 4,486 experienced TMD. Using a random-effects model, the overall pooled prevalence of TMD was estimated at 63.5% (95% *CI*: 50.4–74.9).

The analysis revealed substantial heterogeneity across studies (τ^2 = 2.1669, τ = 1.47, I^2 = 98%, H = 7.41), with the test for heterogeneity being highly significant (Q = 1593.76, p < 0.001).

When stratified by continent, the estimated prevalence of TMD varied. In Asia, the pooled prevalence was 53.9% (95% *CI*: 25.5–79.9), with high heterogeneity (τ^2 = 2.2371, I^2 = 97%). In Europe, the pooled prevalence was 62.2% (95% *CI*: 44.1–77.5), also with substantial heterogeneity (τ^2 = 1.9144, I^2 = 99%). In North America, the prevalence was markedly higher at 98.3% (95% *CI*: 73.7–99.9), with no

Table 1. Meta-regression coefficients with the proportion of co-occurrence of bruxism and temporomandibular disorders (TMD) as an outcome

Predictor	Estimate (log-odds)	*OR*	95% *CI*	*p*-value	Interpretation
Intercept	−2.60	0.07	0.01–0.54	0.010*	The baseline probability of co-occurrence in North America, with no female subjects included in the study sample.
South America	−0.97	0.38	0.09–1.59	0.186	The probability of co-occurrence is 0.38 times that of North America (not significant).
Asia	−2.22	0.11	0.03–0.46	0.003*	The probability of co-occurrence is 0.11 times that of North America (significant difference).
Europe	−2.00	0.14	0.04–0.51	0.003*	The probability of co-occurrence is 0.14 times that of North America (significant difference).
Proportion of females	0.04	1.04	1.02–1.06	<0.001*	A 1% rise in the proportion of females in the study sample increases the probability of co-occurrence by 4.4%.

* statistically significant (p < 0.05, logistic regression test); *OR* – odds ratio; *CI* – confidence interval.

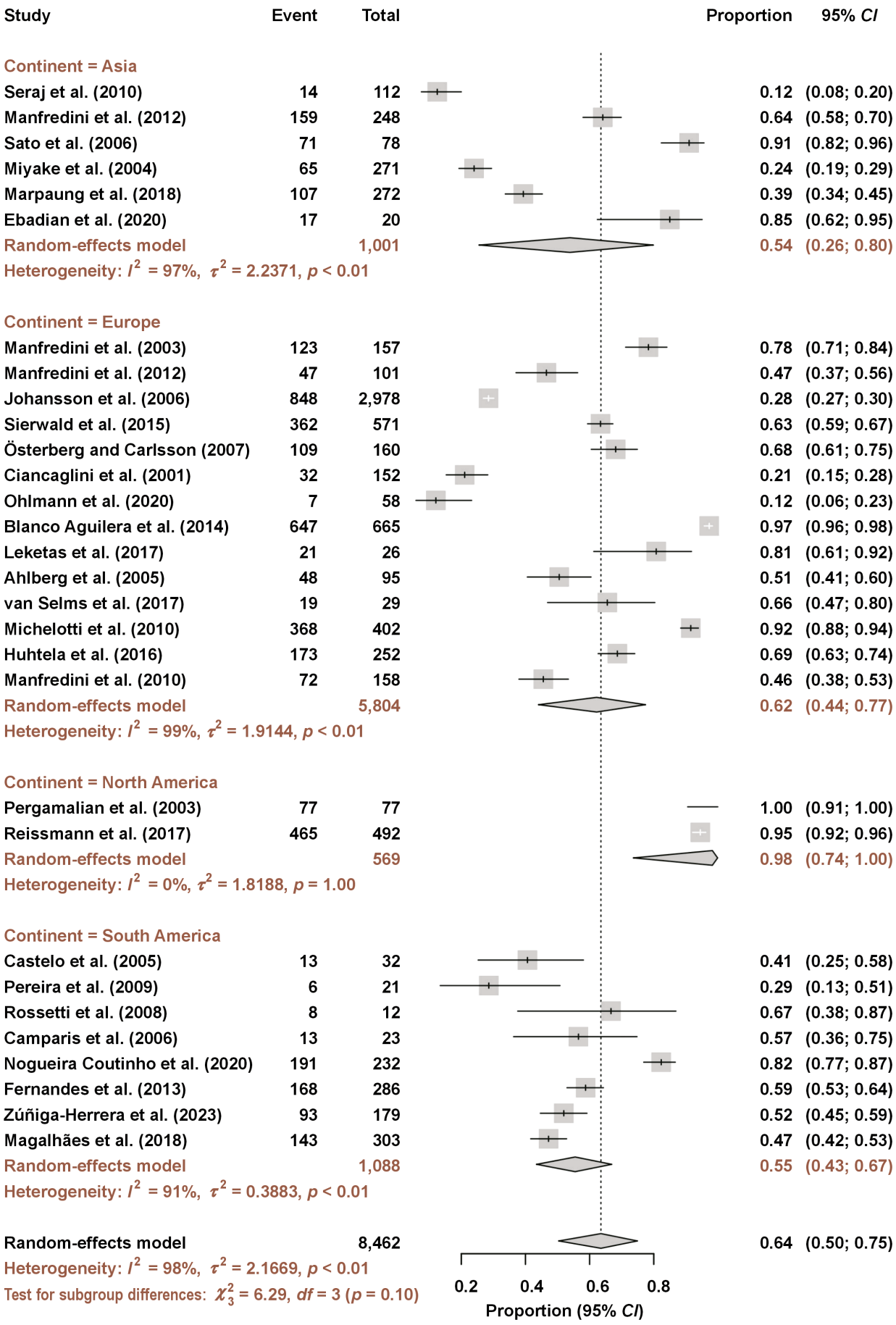

Fig. 6. Forest plot for the overall proportion of temporomandibular disorders (TMD) in patients with bruxism among subgroups by continent

observed heterogeneity (I^2 = 0%). In South America, the pooled prevalence was 55.5% (95% *CI*: 43.5–66.9), with moderate heterogeneity (τ^2 = 0.3883, I^2 = 91%) (Fig. 6).

The test for subgroup differences across continents was not statistically significant (p = 0.098), suggesting that the observed differences in proportions between continents may not represent meaningful variation. However, the notably higher prevalence of TMD in North America compared to other continents warrants further investigation to identify possible methodological or population-specific factors.

In summary, the results highlight a high prevalence of TMD in patients with bruxism globally, but also underscore substantial heterogeneity across studies. The results are visualized by forest plots in Fig. 6.

The influence analysis identified the study by Marpaung et al.[47] as influential. Upon its removal, the majority of the remaining studies contributed to the overall meta-analytic model without exerting disproportionate influence (Figure A.2, supplementary materials; https://osf.io/c38eg).

Standardized residuals predominantly fall within acceptable bounds, with only minor deviations observed for a few studies, suggesting that no extreme outliers affect the model fit. The DFFITS values are generally low, indicating that no single study strongly influences the estimated parameters. However, a few studies showed slightly elevated values, pointing to moderate influence. The Cook's distance remained minimal across studies, with only minor increases observed in a single or 2 studies, which do not substantially alter the pooled effect size. The covariance ratio remained consistent for the majority of studies, except for 1 study[47] that demonstrated a noticeable drop, potentially affecting the precision of the model estimates. Leave-one-out τ^2 and Q statistics demonstrate stability in heterogeneity estimates, with only slight variations for a few studies, indicating that the overall heterogeneity is not driven by a single study. The analysis of hat values and study weights revealed no indications of extreme leverage or disproportion, confirming that the contributions of individual studies are balanced.

Overall, among the remaining 29 studies (30 populations), while a few exhibited mild influence, none exerted an undue impact on the findings of the meta-analysis.

The funnel plot presented in Fig. 7 demonstrates a predominantly symmetrical distribution of studies around the pooled effect size, with no pronounced asymmetry. While a slight imbalance is observed, particularly a tendency for fewer studies with smaller proportions on the left side of the plot, this imbalance is not strongly pronounced.

The distribution of smaller studies, characterized by higher standard errors, appears slightly more dispersed, which is anticipated due to their heightened susceptibility to variability. In contrast, larger studies, characterized by lower standard errors, are more consistent and cluster

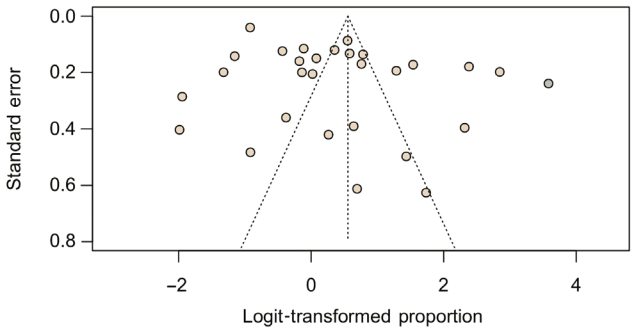

Fig. 7. Funnel plot assessing publication bias in the meta-analysis on the occurence of temporomandibular disorders (TMD) among adult patients diagnosed with bruxism (no labels due to overlap)

closely around the pooled effect size. This pattern is commonly observed in meta-analyses and does not necessarily imply the presence of bias.

Begg's rank correlation test indicated no significant evidence of funnel plot asymmetry (z = 0.23, p = 0.817). The bias estimate was 13.00 (*SE* = 56.05), suggesting that any potential asymmetry is likely due to random variation rather than systematic bias. Simlarly, Peters' linear regression test found no evidence of asymmetry (t (27) = 0.90, p = 0.378). The bias estimate was 25.78 (*SE* = 28.77). This approach, which evaluates the relationship between effect sizes and their precision, further confirms the absence of significant bias.

The results from both tests align with the visual inspection of the funnel plot, indicating that the overall results of the meta-analysis are unlikely to have been influenced by publication bias. While minor visual discrepancies in the funnel plot were identified, they appear to be attributable to random variation rather than systematic bias. Consequently, the meta-analytic conclusions can be interpreted with a high degree of confidence, as there was no substantial evidence to suggest that publication bias had an impact on the pooled estimates.

Discussion

The primary objectives of the study were to estimate the pooled proportions of (1) bruxism (both SB and AB) and TMD co-occurrence and (2) TMD prevalence among individuals with bruxism across continents. The influence of continents on these proportions was assessed using meta-regression, with continents serving as categorical moderators.

The analysis of the co-occurrence of bruxism and TMD revealed a global proportion of 17.1%, with significant differences across continents. The highest proportion was observed in North America (69.8%) and the lowest in Asia (9.4%). In meta-regression, sex was identified as a significant factor, with an elevated proportion of females correlating with an increased likelihood of TMD and bruxism co-occurrence. Conversely, other factors, such as the year

of the study or the average age of the participants demonstrated no statistically significant impact on the variability of the results.

The strengths of the analysis include a large study sample (31 populations, 37,680 individuals) and no substantial evidence of publication bias, as confirmed by a symmetrical funnel plot and statistical tests such as Begg's rank correlation test and Peters' linear regression test. The results emphasize the significance of geographical and demographic factors in understanding the co-occurrence of these conditions. North America, which exhibited the highest proportion, demonstrated moderate heterogeneity (I^2 = 79%), a finding that may be indicative of more consistent diagnostic methods in this region.

Examining the average proportion of TMD among bruxism patients, the global TMD prevalence was found to be 63.5%, which underscores the pervasive nature of TMD in this group. North America exhibited the highest proportion of TMD among patients with bruxism (98.3%), while Asia recorded the lowest prevalence (53.9%). A notable strength of the analysis is the meticulous assessment of the influence of individual studies on the outcomes. The exclusion of the study by Marpaung et al.[47] improved model stability, underscoring the meticulous approach employed. The absence of asymmetry in the funnel plot and the results of Begg's and Peters' tests suggest no significant publication bias, thereby adding confidence to the conclusions.

Previous systematic reviews support the association between bruxism and TMD. For instance, Manfredini and Lobbezoo demonstrated a positive association between bruxism and TMD pain,[7] while Jiménez-Silva et al. suggested that bruxism is likely linked to TMD.[8] Similarly, Mortazavi et al. found a positive relationship,[9] and de Oliveira Reis et al. concluded that children with bruxism are at greater risk of developing TMD.[17] On the other hand, Al-Jewair et al. emphasized that the evidence remains inconclusive, particularly regarding the relationship between TMD and SB.[19]

Given the global TMD prevalence of 63.5% among bruxism patients and the lack of significant publication bias, it can be inferred that bruxism frequently occurs in conjunction with TMD. This finding is consistent with the 2018 consensus, which acknowledged that bruxism alone does not always cause problems. However, when exacerbated by concomitant risk factors, it can contribute to complications.[2] Consequently, bruxism may be an etiological factor for TMD, particularly in the presence of additional contributing factors such as geographical, ethnic,[3,4] genetic,[67,68] or hormonal influences.[4,69]

The study's limitations primarily pertain to the substantial heterogeneity of the results, which complicates interpretation and underscores differences between populations and study methodologies. Diagnostic criteria for TMD, such as the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) and DC/TMD,[1] are globally accepted. However, no standardized criteria for bruxism existed until the Standardized Tool for the Assessment of Bruxism (STAB) was introduced in 2020,[70] with pilot test results published in 2023.[71] Further research using standardized protocols is recommended.

Standardized research protocols and tools are essential for the evaluation of the impact of SB and AB on TMD. A review conducted by Manfredini and Lobbezoo highlighted that studies based on questionnaires or self-reported data often exhibit limited accuracy in assessing SB; however, they consistently indicate a positive association with TMD-related pain.[72] In contrast, studies employing instrumental methods, such as surface electromyography (sEMG) or polysomnography, have frequently demonstrated weaker associations or even a lack of correlation between SB and TMD pain.[72] However, it should be noted that the literature in this field remains inconclusive, and the causal relationship between TMD and bruxism remains a subject of debate. Numerous publications have noted the absence of a definitive link between these phenomena.[2,10–12] A literature review presents both supporting and contradictory arguments regarding this association, necessitating further analysis.[7–9,17–19] Recent polysomnographic studies have confirmed previous findings indicating no association between SB, in its current definition, and TMD. For instance, Sinclair et al. emphasized the lack of such a relationship in their conclusions.[10] Additional studies support these observations. Wieckiewicz et al.[12] noted that the distribution of TMD was similar among patients with SB and non-bruxers, while Smardz et al.[11] found that the occurrence of TMD-related pain is not correlated with the intensity of SB. These findings underscore the need for further research analyzing the impact of bruxism (both SB and AB) on TMD while considering its different subtypes.

However, in a comparative study analyzing which form of bruxism is associated with TMD, Cigdem Karacay and Sahbaz demonstrated that AB is linked to TMJ pain and is also associated with greater functional jaw limitations compared to SB.[13] These examples highlight the complexity of the relationship between SB, AB and TMD.

In light of the findings, it is important to acknowledge the potential influence of cultural differences in emotional expression and pain perception on the outcomes.[73,74] However, to minimize their impact, we applied advanced statistical methods, including subgroup analysis and meta-regression, which enabled us to assess the role of moderators. Additionally, the lack of a shared heterogeneity estimate among the groups (τ^2 not constrained to a common value) enabled the analysis of regional differences, while the random-effects model accounted for the variability between the studies.[50,54] To identify potential systematic biases stemming from cultural differences, we employed funnel plots and asymmetry tests (Begg's rank correlation test and Peters' linear regression test).[58,59]

In the present study, the distinction between AB and SB was not considered. This decision stemmed from

limitations in the data obtained from previously imported reviews. Moreover, the studies included in this meta-regression were primarily based on questionnaires or self-reported data, rather than sEMG or polysomnography.[20–49] While this may introduce some error, the currently imported data from the reviews did not allow for a different approach. Therefore, further research in this area is recommended.

Another significant limitation of the current study is treating TMD as a homogeneous disease entity, despite the fact that TMD encompass various disorders with distinct clinical presentations, pathophysiological mechanisms and demographic distributions. Temporomandibular disorders represent a broad category that includes, among others, myogenous pain, joint–muscle instability and inflammatory joint processes, each with different causes and mechanisms.[1] Failing to differentiate between them may lead to excessive generalization of the results and limit their interpretation; therefore, we recommend caution in analyzing the findings.

A notable limitation of the study is the significant heterogeneity of the included studies, which stems from the use of different assessment methods, including self-reports, clinical exams and quantitative evaluations of bruxism. The incorporation of different diagnostic tools, such as RDC/TMD, DC/TMD and other scales, may result in divergent outcomes, which complicates direct data pooling and increases the risk of error. The detailed information regarding the used tools can be found in the supplementary materials (https://osf.io/c38eg). However, the current scientific data does not permit the application of an alternative approach. The use of a random-effects model partially accounts for this variability; however, it does not eliminate the potential for disparate outcomes across studies. This approach is consistent with previous systematic reviews and meta-analyses.[7–9,17–19] Therefore, further research in this area is recommended.

Additionally, the lack of significant differences across continents (p = 0.098; Fig. 6) may be attributable to limitations in the sample, despite the presence of evident disparities in proportions. For example, in North America, the data was derived from 2 studies, thereby limiting the generalizability of the findings. As evidenced in previous meta-analyses on the prevalence of TMD or bruxism by continent, insufficient data were collected for Africa and Australia, highlighting a need for further research on the co-occurrence of bruxism and TMD in these regions.[3,4]

Conclusions

In summary, the present study suggests that bruxism may contribute to the development of TMD, particularly when additional factors such as geographical, ethnic, genetic, or hormonal influences are involved. The estimated prevalence of the co-occurrence of bruxism and TMD in the global population is 17%. In North America, the co-occurrence of these 2 conditions was 70%, followed by 24% in South America, 14% in Europe, and 9% in Asia. A higher proportion of female participants in study samples significantly increases the likelihood of bruxism and TMD co-occurrence, regardless of the continent. Specifically, a 1% increase in the proportion of females in a study group was associated with a 4.4% increase in the probability of co-occurrence. In Asia, the probability of bruxism and TMD co-occurrence was 89% lower than in North America (the reference group). A similar trend was observed in Europe, where the likelihood was 86% lower than in North America. These findings imply that temporal factors and the average age of participants did not significantly contribute to the observed variability across studies.

The overall prevalence of TMD among patients with bruxism was 63.5%. In North America, this prevalence was the highest (98.3%). This was followed by Europe (62.2%), Asia (53.9%) and South America (55.5%). The notably higher proportion of TMD in North America compared to other continents warrants further investigation to identify potential methodological or population-specific factors.

These findings underscore the importance of geographical and demographic factors in understanding the co-occurrence of bruxism and TMD. The meta-analytic conclusions can be interpreted with a high degree of confidence, as there was no substantial evidence to suggest that publication bias had an impact on the pooled estimates.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets supporting the findings of the current study are openly available in the Open Science Framework at https://doi.org/10.17605/OSF.IO/2AFQR.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Grzegorz Zieliński https://orcid.org/0000-0002-2849-0641
Beata Pająk-Zielińska https://orcid.org/0000-0001-6766-9989
Agnieszka Pająk https://orcid.org/0009-0002-3406-1285
Marcin Wójcicki https://orcid.org/0000-0003-2287-4342
Monika Litko-Rola https://orcid.org/0000-0003-3894-937X
Michał Ginszt https://orcid.org/0000-0002-0800-6103

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Review

Comparative assessment of periodontal conditions between bilateral cleft lip and palate (BCLP) and unilateral cleft lip and palate (UCLP): A systematic review and meta-analysis

Jitesh Wadhwa[A–F], Alpa Gupta[A–F], Puneet Batra[E,F]

Department of Conservative Dentistry and Endodontics, Manav Rachna Dental College, Faridabad, India

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):323–331

Address for correspondence
Jitesh Wadhwa
E-mail: jiteshsds@mrei.ac.in

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on July 26, 2023
Reviewed on August 28, 2023
Accepted on September 5, 2023

Published online on April 8, 2025

Abstract

The existing literature on the periodontal condition in different cleft types is inconclusive and has yielded conflicting results. Therefore, the aim of this systematic review and meta-analysis is to assess and compare the oral health needs of children with bilateral cleft lip and palate (BCLP) with those of children with unilateral cleft lip and palate (UCLP).

Six electronic databases were thoroughly searched for articles published up to June 2022 that directly compared the periodontal condition of BCLP patients with that of UCLP patients. A meta-analysis was conducted using the random-effects model with inverse variance weighting. The literature search yielded 858 articles, out of which 58 studies were selected for a full-text review. Finally, 5 articles, which compared 86 BCLP individuals with 132 UCLP patients across 3 continents, were evaluated. The selected papers compared gingival and periodontal parameters, including the plaque index (PI), the gingival index (GI), periodontal probing depth (PPD), and clinical attachment loss (CAL).

The meta-analysis revealed a significant difference in CAL on the facial side in BCLP individuals (mean difference: −0.44, 95% confidence interval (*CI*): 0.27–0.61, $Z = 5.07$, $p < 0.0001$). The remaining parameters did not reveal any significant differences between the 2 groups.

In light of the established correlation between cleft lip and palate morbidity and surgical interventions on gingival and periodontal health, these factors must be incorporated into treatment planning.

Keywords: cleft palate, plaque index, periodontal diseases, gingival index, cleft lip

Cite as
Wadhwa J, Gupta A, Batra P. Comparative assessment of periodontal conditions between bilateral cleft lip and palate (BCLP) and unilateral cleft lip and palate (UCLP): A systematic review and meta-analysis. *Dent Med Probl.* 2025;62(2):323–331. doi:10.17219/dmp/171896

DOI
10.17219/dmp/171896

Highlights

- Anatomical cleft characteristics, malaligned teeth and skeletal discrepancies contribute to periodontal attachment loss in cleft patients.
- The systematic review revealed higher attachment loss on mesial, facial and palatal surfaces of maxillary canines.
- Treatment planning for cleft lip and palate patients should consider periodontal conditions to ensure optimal oral health outcomes.

Introduction

Cleft lip and palate is one of most prevalent congenital malformations within the head and neck region,[1–4] with incidence rates of 1:700 live births. Epidemiological studies have demonstrated that the prevalence of cleft anomalies may vary depending on geographical location, socioeconomic status and racial background.[5] The American Indian population exhibited the highest prevalence rates of 2.62 per 1,000 live births, followed by the Japanese, Chinese, and White populations with 1.73, 1.56, and 1.55 per 1,000 live births, respectively. The Black population exhibited the lowest rate of 0.58 per 1,000 live births.[6] Furthermore, data spanning a 5-year period revealed that the overall congenital anomaly rate increased in the United States and decreased internationally.[7]

Orofacial clefts represent a heterogeneous group of congenital malformations with different morphologic presentations, ranging from cleft lip alone to complete unilateral cleft lip and palate (UCLP), bilateral cleft lip and palate (BCLP), and isolated clefts of soft palate, resulting from the lack or incomplete fusion of the medial nasal process with the maxillary process during the first stage of embryonic development.

Cleft lip and palate is also associated with a number of syndromes, such as Treacher Collins syndrome, Pierre Robin syndrome and DiGeorge syndrome, which have been linked to a variety of factors, including increased maternal age, tobacco smoking and alcohol consumption. Although the precise etiology remains unclear, mutations in the *PAX9*, *TGF-β*, *IRF*, and *MSX1* genes play a pivotal role in fetal development. Unilateral clefts account for 75% of all cases, while 25% are bilateral. In unilateral clefts, the left side is more frequently affected. The majority of dental anomalies in CLP patients occur in the anterior region of the maxilla. This observation may be related to the surgical procedures performed in this region during the process of tooth bud formation.[8]

Individuals with cleft lip and palate often experience impaired orofacial functions, including speech, deglutition and oral health. Consultations with patients who present with cleft anomalies commence immediately after birth, and the initial treatment begins during the first month after childbirth. Cleft palate associations worldwide, including the American Cleft Palate Craniofacial Association (ACPA), concur that the management of these patients is best provided by a multidisciplinary team of specialists, including oral and maxillofacial surgeons, pediatricians, orthodontists, speech therapists, prosthodontists, pedodontists, as well as medical professionals such as pediatricians, speech therapists, phoniatricians, and laryngologists.[9,10]

The development of carious lesions and periodontitis is increased in individuals with cleft lip and palate.[11,12] Even before the complete closure, the soft tissue folds complicate access to target areas with conventional cleaning techniques and may serve as a habitat for putative pathogens. This, in turn, increases the risk of intraoral translocation of pathogens, leading to periodontal infection.[13] Authors have reported an increased prevalence of caries and periodontal breakdown rates among UCLP and BCLP patients, respectively.[3,14,15] Dental and arch segment irregularities, orthodontic appliances and the presence of Simonart's band, a soft tissue band that connects the cleft gap of the base of the nostril or the margin of the alveolus after cleft closure,[16] collectively contribute to the progression of periodontal disease.[3,14,15,17]

A substantial body of epidemiological research has demonstrated that control subjects exhibited optimal oral health status when compared to subjects with cleft palate. There is a paucity of research regarding oral health status among different cleft types. To date, no systematic review has explored the periodontal status of individuals with different cleft types. Therefore, the present systematic review aimed to assess the periodontal status of patients with BCLP compared to those with UCLP.

Material and methods

This systematic review was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Fig. 1) and the PICOS framework, as follows: Patients: children, adolescents and adults with cleft lip and palate; Intervention/exposure: presence of BCLP; Control: UCLP group; Outcome: periodontal status; Study design: observational and cross-sectional studies (Table 1). Two authors (JW and AG) independently performed the data extraction after selecting the articles relevant to the review. Any disagreements between the authors were resolved by the third reviewer (PB).

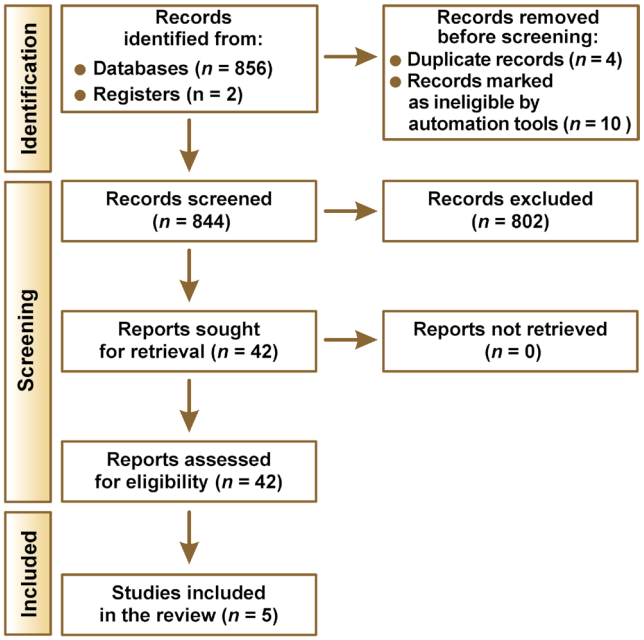

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flowchart of the study

The collected data was organized in a tabular form consisting of study design, sample size, participants' age and gender, dentition type, cleft type, group matching, study outcomes, and utilized periodontal indices (Table 2). The study has been registered in Open Science Framework (doi:10.17605/OSF.IO/KNJZE).

Search strategy

A structured literature search of PubMed®, Scopus, Cochrane, Web Of Science, Cochrane Central Register of Controlled Trials (CENTRAL), and OpenGrey databases was conducted. Additionally, unpublished literature was retrieved from Clinical Trials Registry – India. The search was limited to articles published up to February, 2023. Reference lists of the selected articles were also screened using cross-referencing. The search utilized the following Medical Subject Headings (MeSH): "cleft lip"; "cleft palate"; "periodontal status"; "attachment loss"; "oral hygiene". These terms were combined with Boolean operators (AND and OR) to formulate a search strategy that was pertinent to the review question. The selection of MeSH terms from the top of the MeSH tree hierarchy was deliberate, ensuring the inclusion of subheadings within the search.

Screening and selection

The results obtained after the implementation of the search strategy were transferred into the online tool Rayyan (https://www.rayyan.ai), which enabled the authors to screen the titles and abstracts of the articles. The selection criteria encompassed observational and cross-sectional studies that compared 2 groups and presented the data quantitatively. The analysis included original research that compared the periodontal evaluation of BCLP with that of UCLP, with UCLP serving as the control. Studies involving bone grafts, dental anomalies, clefts in syndromic patients, as well as case reports, case series, and letters to the editor were excluded from the analysis. Reference lists of pertinent articles and gray literature (OpenGrey) were searched to identify potentially relevant papers that might have been missed during the previous steps. Studies reflecting indirect data, qualitative data, and studies without the control group were excluded, but the references of the articles were reviewed to identify any potential studies.

Objectives

This paper provides a comprehensive insight into the periodontal conditions that are prevalent among individuals with BCLP around the world. The infrastructure demands and treatment needs of these patients differ significantly from those with UCLP. This knowledge can assist healthcare centers and governing bodies in the formulation of policies for the care of cleft individuals across different age groups.

Results of the search

The literature search yielded 858 articles (Fig. 1). The selected article list was transferred to the online tool

Table 1. PICOS format and research question

PICOS	Description
Patients	children, adolescents and adults with cleft lip and palate who have or have not undergone surgical intervention and who have not been diagnosed with any syndrome
Intervention/exposure	presence of BCLP
Control	UCLP group
Outcome	assessment of the periodontal condition using established indices and protocols
Study design	observational and cross-sectional studies
Research question	Are there any differences in the periodontal condition of BCLP individuals compared to that of UCLP individuals?

BCLP – bilateral cleft lip and palate; UCLP – unilateral cleft lip and palate.

Rayyan for the purpose of sorting and selecting the relevant articles based their titles and abstracts. Following the removal of ineligible records and duplicates (n = 14), a total of 802 studies were excluded. After a full-text review, a total of 42 studies were included. The final review included 5 articles that met the inclusion criteria and were selected for meta-analysis. The detailed characteristics of studies that met the inclusion criteria are outlined in Table 2.

Table 2. Characteristics of the studies included in the review

Study	Country	Design	Sample size	Control group	Cleft surgery	Mean age [years]	Dentition examined	Method of outcome assessment	Parameters assessed	Results
Sudhakar et al. 2007[19]	India	case–control	20 BCLP patients	20 UCLP patients	not reported	15	not reported	clinical examination	PI, SBI, PPD, CAL	With the exception of SBI, all parameters exhibited significantly higher levels in BCLP patients.
Eldeeb et al. 1986[20]	USA	cross-sectional	26 cleft patients (17 M, 9 F, 8 BCLP, 18 UCLP)	29 non-cleft patients (11 M, 18 F)	patients have undergone alveolar bone grafting using an autogenous iliac crest graft which was covered with either a mucogingival or a mucobuccal flap using a surgical technique described by Broude and Waite	BCLP: 16.8 UCLP: 16.2	permanent (maxillary canine and 6 Ramfjord teeth)	clinical examination	PI, GI, PPD, CAL, width of attached gingiva in the canine region	Patients with cleft palate demonstrated higher PI values. No significant differences were observed in GI, PPD and CAL.
Gaggl et al. 1999[21]	Austria	cross-sectional	50 cleft patients (30 UCLP, 20 BCLP)	30 UCLP patients	not reported	BCLP: 21.4 UCLP: 18.9	permanent	clinical examination	CAL, API, SBI, pathologic mobility	Patients with cleft palate had elevated SBI scores. The BCLP group exhibited a higher prevalence of periodontal damage, particularly in teeth adjacent to the cleft area.
Hazza'a et al. 2011[22]	Jordan	cross-sectional	98 cleft patients (52 UCLP, 46 BCLP)	98 non-cleft patients	not reported	12 ±6.3	primary and permanent	clinical examination	PI, GI, DMFT, DMFS	The prevalence of plaque and gingivitis was higher in the cleft group. The BCLP group exhibited a higher incidence of gingivitis.
Pisek et al. 2014[23]	Thailand	cross-sectional	68 cleft patients (34 M, 34 F, 20 BCLP, 36 UCLP)	118 non-cleft patients (48 M, 70 F)	not reported	BCLP: 11.9 UCLP: 11.9	primary and permanent	interview and oral examination	PI, GI, DMFT, DMFS, quality of life	The examined patients demonstrated high DMFT, PI and GI scores, which had an impact on their ability to speak and smile. No significant differences in caries were observed in the primary dentition.

M – male; F – female; PI – plaque index; GI – gingival index; DMFT – number of decayed, missing and filled permanent teeth; DMFS – number of decayed, missing and filled surfaces; PPD – periodontal probing depth; CAL – clinical attachment loss; API – approximal plaque index; SBI – sulcus bleeding index.

The majority of the analyzed studies were of a cross-sectional nature, while 1 study was of a case–control design. The studies reflected the data in the form of subset parameters of periodontal assessment of individuals with BCLP and UCLP. The articles assessed the periodontal condition of 86 BCLP individuals, with a mean age of 15 years. The studies were conducted in Jordan, Austria, the United States, Thailand, and India. None of the selected studies incorporated syndromic patients, a factor that could potentially introduce confounding variables. The studies have divided the sample according to the cleft type. The male-to-female ratio ranged from 30:70 to 62:38 in the experimental group, and it was 40:60 in the control group of UCLP patients. With regard to the cleft surgery, the presence or absence of surgery, and the time elapsed since surgery were reported in only 1 study.

Characteristics of the selected studies

The selected studies have evaluated the periodontal condition using various parameters. A study by Ali and Mazin selected teeth representative of the overall periodontal status for the individual patient, according to Ramfjord, namely maxillary right first molar, maxillary right canine, maxillary left central incisor, maxillary left canine, maxillary left first premolar, mandibular right central incisor, and mandibular right first premolar.[18] Out of the 5 studies, 4 evaluated hygiene by means of the plaque index (PI),[19,20,22,23] 1 study utilized the approximal plaque index (API),[21] 3 evaluated gingival health by means of the gingival index (GI),[20,22,23] 2 evaluated periodontal condition using the sulcus bleeding index (SBI),[19,21] and 2 by means of periodontal probing depth PPD).[19,20] Additionally, 3 papers evaluated clinical attachment loss (CAL),[19–21] 1 study measured pathologic mobility,[21] and 1 study assessed patients' quality of life.[23] A study by Gaggl et al. evaluated the periodontium after orthodontic treatment.[21] However, this evaluation may not accurately reflect the true state of the periodontium due to the potential adverse effects of orthodontic brackets and dentoalveolar expansion on oral hygiene, particularly in the cleft area that has undergone multiple surgical procedures throughout its lifetime.

Table 3. Newcastle–Ottawa Scale (NOS) adapted to assess the quality of cross-sectional studies for the systematic review

Study	Selection	Comparability	Outcome	Quality score
Sudhakar et al.[19]	**	**	*	5
Eldeeb et al.[20]	****	0	**	6
Gaggl et al.[21]	****	*	***	8
Hazza'a et al.[22]	****	**	**	8
Pisek et al.[23]	****	**	**	8

Quality assessment

A set of 4 quality assessment criteria was established based on the Newcastle–Ottawa Scale (NOS) adapted for the evaluation of the quality of cross-sectional studies for the systematic review.[24] Table 3 presents a modified version of the NOS scale that was used to assess study quality. The criteria encompassed a series of assessments, including the extent to which the study has outlined the selection criteria for participants, control, ascertainment of exposure (disease), the comparability with respect to study design or analysis, and the control of confounding factors. Lastly, the outcome, whether structured or self-reported, was assessed. The study and control groups were matched in all studies.

Results

The oral hygiene of the participants was evaluated using PI, API[25] and GI. Gingival condition was assessed using the gingival index tool, which is based on the criteria outlined by Silness and Loe.[26] The results were based on the assessment of the mean difference in PI and GI scores between cleft groups (mean difference: 0.14 (0.01–0.27)). However, the comparison of the studies did not reveal any statistically significant differences ($Z = 2.09$, $p = 0.04$). As illustrated in Fig. 2 and Fig. 3, the BCLP group exhibits a favorable positioning within the forest plots.[20,23] The heterogeneity between the studies was found to be low ($I^2 = 40\%$) when studies evaluating gingival indices were compared (Fig. 2). However, a considerable heterogeneity was identified in studies assessing plaque condition ($I^2 = 83\%$) (Fig. 3).

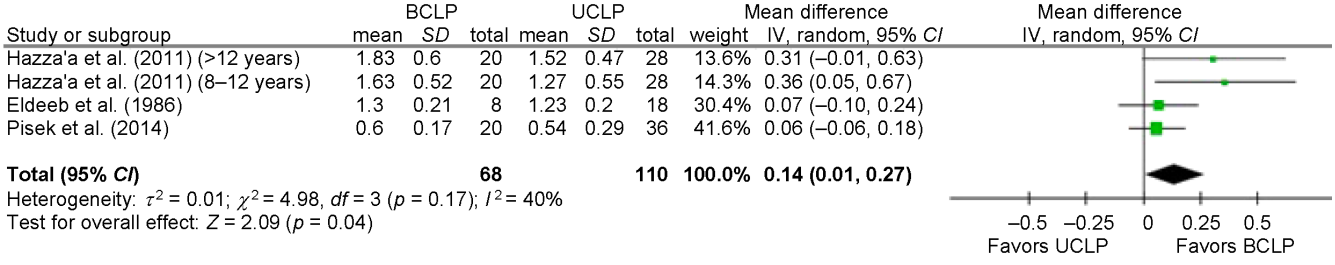

Fig. 2. Forest plot comparing the gingival index (GI) between bilateral cleft lip and palate (BCLP) and unilateral cleft lip and palate (UCLP) patients

SD – standard deviation; *CI* – confidence interval; *df* – degrees of freedom.

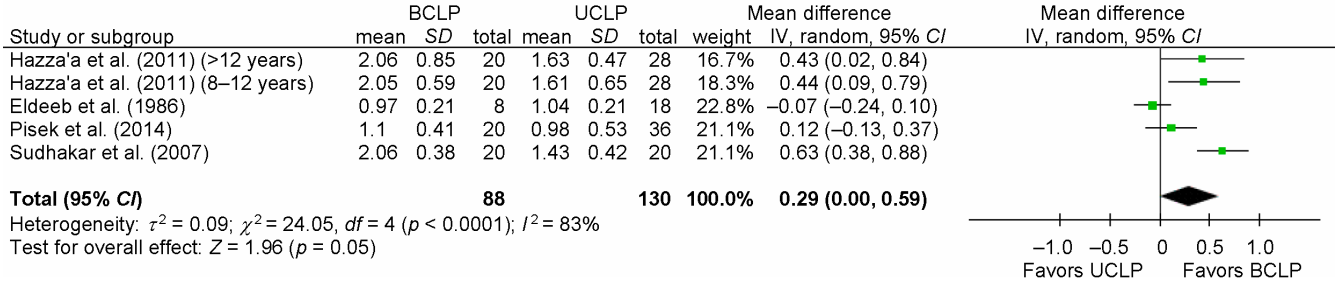

Fig. 3. Forest plot comparing the plaque index (PI) between BCLP and UCLP patients

Periodontal evaluation – CAL, SBI, PPD

The periodontal index developed by Silness and Loe[26] and CAL[27] were used to assess the periodontium. The selected studies evaluated the periodontal status of the maxillary arch in the anterior region, posterior region, and teeth adjacent to the cleft. However, these factors were not considered in the meta-analysis because the data could not be compared. Therefore, the present study considered CAL on all surfaces of the maxillary canine at the cleft side, namely the mesial, facial, palatal, and distal surfaces. A statistically significant difference was identified in CAL on the facial surface of BCLP (mean difference: –0.44, 95% confidence interval (*CI*): –0.61–-0.27, $Z = 5.07$, $p < 0.00001$), and a low level of heterogeneity was identified ($I^2 = 0\%$) (Fig. 4). The assessment of publication bias was not feasible due to the limited number of studies available. On the 3 remaining surfaces, the periodontal condition in the BCLP group did not differ from that in the UCLP group, as depicted by their forest plots (Fig. 5–7).

Discussion

The current review focuses on the periodontal assessment among different cleft types. Previous studies have attempted to reflect the prevalence of caries, skeletal

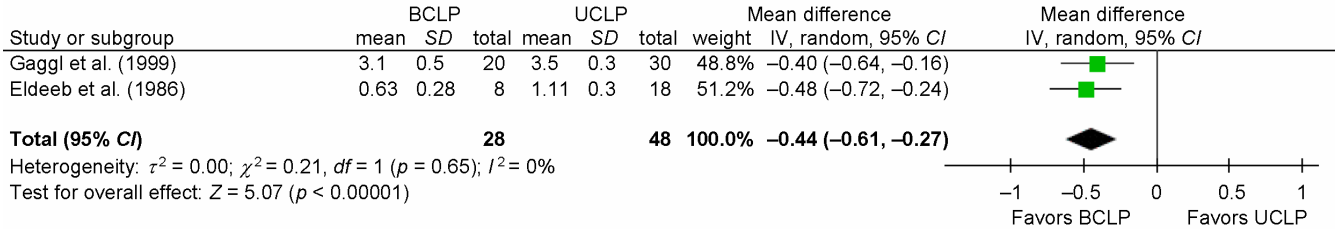

Fig. 4. Forest plot comparing clinical attachment loss (CAL) between BCLP and UCLP patients on the facial surface of maxillary canines

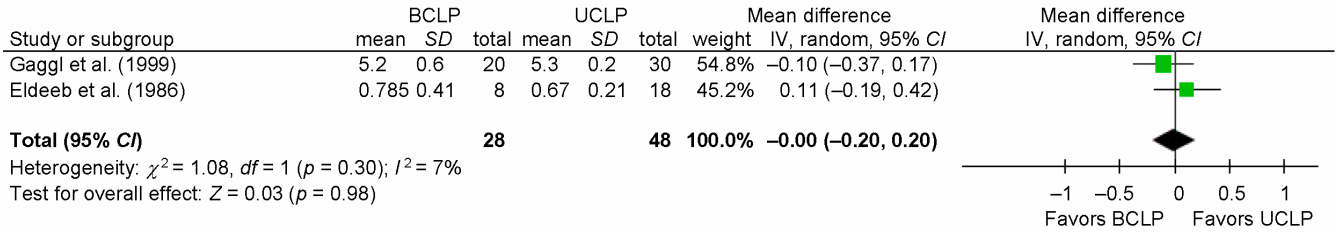

Fig. 5. Forest plot comparing CAL between BCLP and UCLP patients on the distal surface of maxillary canines

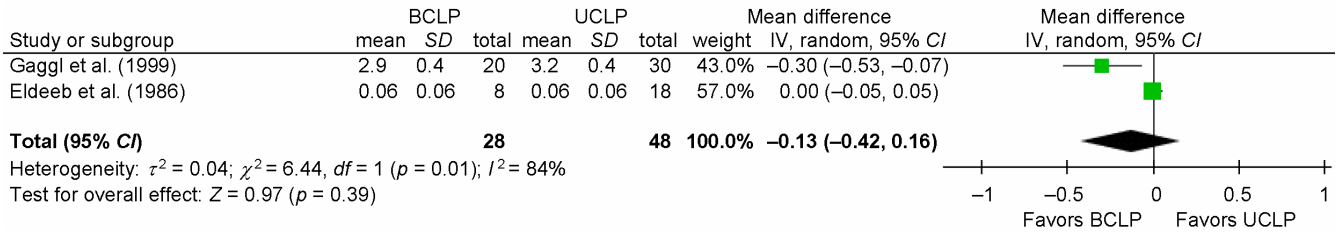

Fig. 6. Forest plot comparing CAL between BCLP and UCLP patients on the palatal surface of maxillary canines

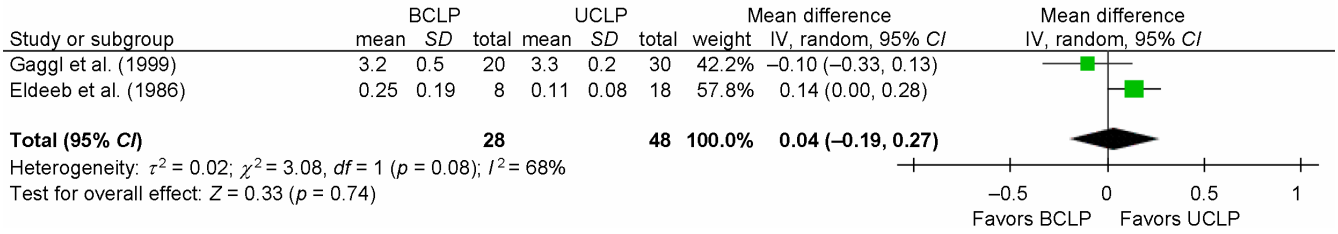

Fig. 7. Forest plot comparing CAL between BCLP and UCLP patients on the mesial surface of maxillary canines

morphology,[28] periodontal status, and quality of life[12,29,30–35] in cleft individuals. However, none of the studies have analyzed the findings according to the type of cleft. A comparison of caries prevalence and periodontal condition between cleft and non-cleft groups is not feasible due to the non-comparability of the groups, as they have undergone different surgical interventions with varying levels of morbidity at different stages of development. Additionally, the treatment approach differs between cleft types, underscoring the need to identify existing periodontal problems in BCLP to facilitate the development of customized treatment planning.[36]

Marzouk et al. conducted a systematic review to determine whether individuals with non-syndromic orofacial clefts (OCs) had more dental abnormalities (DAs) than those without OCs.[37] The outcomes proved that individuals with OCs are more likely to present with a range of DAs than their unaffected peers. Statistically significant associations were observed between OCs and supernumerary teeth, developmental enamel defects, malposition and/or transposition, rotation, and impaction.[37]

In the present systematic review, 5 studies were identified, and data from these studies was assembled for the comparison of CAL, gingival indices and periodontal indices across different surfaces of canine teeth. The review encompassed a total of 86 individuals with BCLP and compared them to 132 UCLP patients. The data from the selected studies reflected that the 2 groups have comparable gingival and periodontal indices. However, significantly higher CAL values were reported in the BCLP groups. The meta-analysis revealed a significantly higher mean CAL on the facial aspect of canine teeth.

The observed discrepancy in CAL may be statistically significant, but not clinically significant, due to the potential role of scar tissue. A study by Lucas et al. reported no significant difference in PI between cleft and non-cleft individuals, as compared to studies performed by other authors.[12] This difference could be attributed to a small sample size. Additionally, participants with different cleft types received multidisciplinary care starting at an early age.[12]

A study by Paul and Brandt reported better dental health in participants in which cleft or palate was not involved.[38] Secondly, the surgical technique employed for uncovering the canines contributed to attachment loss. However, the absence of documentation regarding the exact technique used made this difficult to verify.

Limitations

The results of the review should be interpreted with caution due to the limited number of studies that reflect the data. Studies in languages other than English were not considered, and the articles were not searched manually. These factors could introduce a significant confounding factor into the study. Additionally, the data was not organized based on sex, as the number of studies was limited and the sample size was small, thus dividing the sample was not feasible. Consequently, the funnel plots were not created.

The included articles have followed the methodological criteria laid down by GI, PI and CAL indices. The studies have not elaborated on intraoperative errors, which could have affected the results of the present study.

Within the limitations of this review, the available evidence suggests that, due to the increased morbidity observed in the BCLP group, these individuals may exhibit slightly poorer PPD and CAL compared to the UCLP group. The clinical significance of this increase remains uncertain.

Conclusions

The primary factors contributing to attachment loss include the anatomical characteristics of the cleft area, maligned teeth, and discrepancies in the skeletal base relationship. In addition, developmental aspects related to surgical repair, surgical bone grafting procedures, hypoplastic defects, and scarring, in conjunction with various phases of orthodontic treatment, may restrict access to adequate oral hygiene and predispose patients to plaque accumulation. The present study systematically reviewed the extant literature, encompassing 5 studies that compared the periodontal parameters among individuals with cleft palates. The analysis revealed a significantly higher prevalence of attachment loss on the mesial, facial and palatal surfaces of canines, with grafted gingiva resulting in surgical uncovering rather than orthodontic intervention.

Ethics approval and consent to participate

Not applicable.

Data availability

The datasets supporting the findings of the current study are openly available in Open Science Framework at https://osf.io/knjze (doi:10.17605/OSF.IO/KNJZE).

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

ORCID iDs

Jitesh Wadhwa https://orcid.org/0000-0003-2834-0608
Alpa Gupta https://orcid.org/0000-0001-8047-5054
Puneet Batra https://orcid.org/0000-0003-4201-4235

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Review

Skeletal and dental changes after bone-borne versus tooth-borne surgically assisted rapid palatal expansion in subjects with maxillary transverse deficiency: A systematic review and meta-analysis

Leelan Kanwal[A–E], Hafsa Qabool[A–D], Wafa Idrees[B–D], Rashna Hoshang Sukhia[C,E,F], Mubassar Fida[E,F]

Department of Surgery, Aga Khan University Hospital, Karachi, Pakistan

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):333–341

Address for correspondence
Rashna Hoshang Sukhia
E-mail: rashna.aga@aku.edu

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on July 23, 2023
Reviewed on July 31, 2023
Accepted on August 19, 2023

Published online on April 8, 2025

Abstract

The objective of the study was to determine the difference in skeletal and dental effects of bone-borne (BB) versus tooth-borne (TB) surgically assisted rapid palatal expansion (SARPE) in subjects with maxillary transverse deficiency (MTD).

The present review included randomized controlled trials (RCTs), non-RCTs and cohort studies. A systematic search was conducted in online databases (i.e., PubMed®, Dentistry & Oral Sciences Source, CINAHL Plus, and Cochrane Central Register of Controlled Trials (CENTRAL)) for articles published up to January 2023. The outcome was estimated using the weighted average difference and 95% confidence intervals (*CI*s). The heterogeneity of the studies was assessed using Cochran's heterogeneity test (I^2 test). The meta-analysis was conducted using the RevMan software, v. 5.3.5.22.

The qualitative and quantitative synthesis incorporated 7 articles that satisfied the inclusion criteria. The skeletal and dental expansion was assessed pre- and post-expansion in 249 patients who underwent SARPE with BB and TB appliances. Five studies were included in the meta-analysis to measure skeletal expansion in the first premolar and first molar regions. The analysis revealed no statistically significant differences between the study groups (mean difference: −0.16; 95% *CI*: −0.34, 0.67). To measure dental expansion, 7 studies were included in the meta-analysis, and no significant differences were observed between them (mean difference: −0.29; 95% *CI*: −0.77, 0.19).

This systematic review and meta-analysis revealed no differences in skeletal and dental expansion in patients who underwent SARPE with BB and TB appliances.

Keywords: surgically assisted rapid palatal expansion, SARPE, tooth-borne, bone-borne

Cite as
Kanwal L, Qabool H, Idrees W, Sukhia RH, Fida M. Skeletal and dental changes after bone-borne versus tooth-borne surgically assisted rapid palatal expansion in subjects with maxillary transverse deficiency: A systematic review and meta-analysis. *Dent Med Probl.* 2025;62(2):333–341. doi:10.17219/dmp/171393

DOI
10.17219/dmp/171393

Highlights

- The study quantifies skeletal and dental expansion to support appliance selection in surgically assisted rapid palatal expansion (SARPE) treatment planning by oral surgeons and orthodontists.
- The analysis included 7 studies that met predefined inclusion criteria for qualitative and quantitative assessment.
- There was no statistically significant difference in skeletal and dental expansion between bone-borne and tooth-borne appliances.

Introduction

In orthodontic clinics, maxillary transverse deficiency (MTD) is a frequently observed condition.[1–3] Proffit and White stated that 30% of adult orthodontic patients had MTD.[4] The reported prevalence of MTD is 8–18% in children and 10% in adults.[5–7] In Argentina, the prevalence of MTD in primary dentition was found to be 0.3%.[8] Gungor et al. reported a prevalence of 15.6% in the Turkish population,[9] while Nainani and Relan documented a prevalence of 5.5% in the Indian population.[10] Additionally, a study conducted by van Wyk and Drummond revealed a prevalence of 10.4% within a South African population.[11] Dacosta and Utomi further substantiated that a higher incidence of MTD was evident among women compared to men.[12]

Maxillary transverse deficiency, with its resulting sequelae, can present with decreased intercanine and intermolar widths, and unilateral or bilateral crossbites with deep and narrow palatal vaults.[13,14] It can further lead to crowding, excessive buccal corridors, non-carious cervical wear of teeth, periodontitis, and imbalances in facial musculature.[15–17] To avoid these unfavorable manifestations and achieve a stable occlusion, it is essential to provide patients with a normal transverse skeletal relationship.[18]

In adolescents, rapid maxillary expansion (RME) has proven to be the most successful orthodontic approach for addressing transverse maxillary discrepancies.[19,20] This therapeutic method involves opening the midpalatal suture and widening the maxillary arch.[21] However, with increasing age, the fusion of the midpalatal suture and adjacent articulations leads to resistance to the forces of expansion appliances.[22] The utilization of these appliances in adults can result in a greater impact on dental than skeletal effects. Furthermore, these appliances can cause several side effects, including dental crown tipping, dehiscence, periodontal damage, root resorption, and instability of the achieved results.[23,24] In non-growing individuals, surgically assisted rapid palatal expansion (SARPE) is indicated to overcome the hindrance from the ossified sutures and resulting side effects.[25]

Surgically assisted rapid palatal expansion is a reliable technique for the treatment of MTD in skeletally mature patients.[25] In this procedure, an osteotomy is performed, and the sutures restricting the expansion are released. Subsequently, an expander is placed and activated until the achievement of the desired outcomes.[26] The expanders used after SARPE to perform activation depend on the preference of the practitioner. These appliances can be broadly categorized into bone-borne (BB) and tooth-borne (TB) expanders.[27]

After the SARPE procedure, TB appliances are banded to the teeth without requiring additional invasive procedures. They provide satisfactory results.[28] However, studies have demonstrated that when these conventional TB expanders are used, the expansion forces are applied through the teeth, thereby complicating the control of the relapse of the expanded segments during the consolidation period.[29,30] Moreover, they can often result in dental tipping, alveolar bone dehiscence and periodontal damage.[29] Mommaerts introduced the BB SARPE technique to avoid these undesired side effects.[31] The BB expanders directly transmit the expansion forces to the palatal region, causing more skeletal and less dental effects.[32] Additionally, they can prevent the relapse of the expanded bony segments in the consolidation period.[33]

During the literature search, we identified several systematic reviews that evaluated skeletal and dental effects following SARPE.[26,34] Upon conducting an in-depth review of these studies, it became evident that the available research lacked comprehensive data to assess expansion in the anterior region. Moreover, the studies that were included in previous reviews did not have a comparative design. Consequently, we conducted this systematic review while taking into account the limitations of previous studies to thoroughly evaluate the qualitative and quantitative aspects of studies within the scope of our review. Few randomized controlled trials (RCTs) that have evaluated skeletal and dental changes after BB versus TB SARPE have contradictory or unclear results.[28,32] Hence, this systematic review and meta-analysis was performed to generate strong evidence.

Objectives

The current review aimed to collect data and develop high-quality evidence. The review question was as follows: Is there a difference in skeletal and dental effects of BB compared to TB SARPE in subjects with MTD?

Material and methods

Eligibility criteria

The review question was developed in accordance with the PICOS (Population, Intervention, Comparison, Outcomes and Study design) criteria. The population comprised orthodontic patients who underwent SARPE, while BB expanders served as the intervention group and TB expanders were designated as the comparison group. The outcomes assessed included skeletal and dental changes.

The present review encompassed RCTs, non-RCTs and cohort studies. Case reports, case series, reviews, case–control, single-arm longitudinal, and animal studies were excluded from the analysis.

Search strategy

Major health databases (i.e., PubMed®, CINAHL Plus, Cochrane Central Register of Controlled Trials (CENTRAL), Dentistry & Oral Sciences Source) were extensively searched for articles published up to January 2023. Additionally, unpublished and grey literature, along with Google Scholar, were searched manually. The following Medical Subject Headings (MeSH) were used: ("Orthodontics"[MeSH] orthodontic*OR dental OR dentistry OR) AND (Skeletal OR soft tissue OR airway OR suture opening comparison) AND (Surgically assisted rapid maxillary expansion OR mini-screw assisted rapid palatal expansion OR SARPE OR SARME OR surgically assisted rapid palatal expansion OR MARPE OR bone-anchored rapid palatal expansion).

Study selection and data extraction procedure

The systematic review included RCTs, non-RCTs and cohort studies that evaluated primary outcomes of skeletal and dental changes. The obtained results were transferred after the literature search to EndNote™ X 9.2 software (Clarivate, Philadelphia, USA) for citation management. Two independent authors (LK and HQ) performed a two-phase selection process to scrutinize the results. In the 1st phase, titles and abstracts of the articles were reviewed. The full texts of studies that remained after the initial screening were then evaluated in the 2nd phase. In the event of a discrepancy between the 2 authors, the 3rd author (WI) was consulted. The 3rd author repeated the selection process. The results revealed a high degree of agreement across all examinations (intraclass correlation coefficient (ICC) = 0.88). The data was entered into a standardized proforma. The veracity of the data was assessed, and any inconsistencies were resolved by the reassessment of the original studies (Fig. 1).[35]

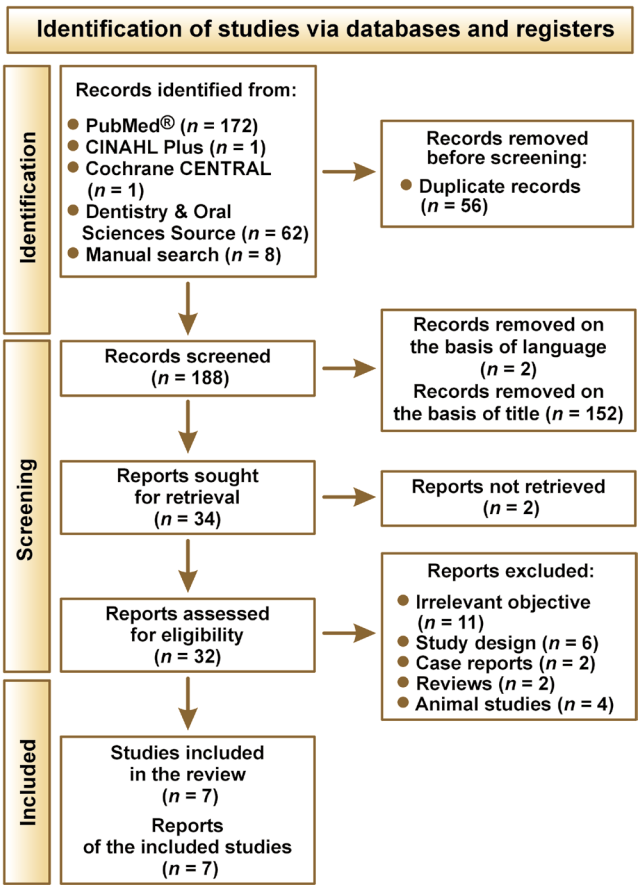

Fig. 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses)35 flowchart of the study

CENTRAL – Central Register of Controlled Trials.

Effect measures and synthesis of results

The data analysis encompassed a systematic assessment of skeletal and dental changes, with a focus on the canine, first premolar and first molar regions. The outcomes were based on the assessments conducted in individual studies. The results of these studies were then meticulously analyzed with regard to the aforementioned regions. For quantitative data, a meta-analysis was performed using Review Manager (RevMan), v. 5.4 (Cochrane Collaboration, London, UK). The I^2 statistic was used to determine the heterogeneity between the studies. Random- and fixed-effects models were utilized for the analysis of the summary effect.

Assessment of the risk of bias in individual studies

The Cochrane's risk of bias (RoB 2.0) assessment tool[36] was used for the assessment of RCTs. The RoB 2.0 tool comprises multiple domains that allow for the classification of RCTs into low, unclear and high risk of bias categories. The Newcastle–Ottawa scale[37] was utilized to determine the quality of a non-RCTs and cohort studies.

Assessment of certainty

The GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach[38] was utilized to ascertain the overall strength of evidence of the systematic review after assessing the individual included studies.

The GRADE tool evaluates the included studies based on their research design, risk of bias, inconsistencies, and any imprecision or indirectness for each outcome. The tool categorizes the outcomes into extremely low, low, moderate, or high-quality evidence (Table 1).

Results

Selection process and characteristics of the included studies

The initial search yielded 244 citations. After the elimination of duplicates, the number of citations was reduced to 188. Further scrutiny was conducted on the basis of titles, abstracts and language, which further reduced the number of articles to 34. Following a thorough examination of the full texts of the papers, 7 articles were deemed suitable for inclusion in the systematic review and meta-analysis. The selection process is outlined in Fig. 1. Of the 7 articles selected for inclusion, there were 4 RCTs, 1 non-RCT and 2 cohort studies. All the included studies compared the dental and skeletal changes after SARPE with BB and TB expanders. A comprehensive summary of the individual studies is presented in Table 2.

Assessment of the risk of bias within and across studies

The risk of bias was assessed for the RCTs using the 5 domains of the RoB 2.0 tool. All RCTs exhibited a low risk of bias in the 3rd and 5th domains. For the 1st and 4th domains of the RoB tool, studies by Koudstaal et al.[39] and Kayalar et al.[40] exhibited a low risk. However, studies by Landes et al.[32] and Zandi et al.[28] had a high risk of bias in these domains. Overall, 50% of the studies[39,40] had some concerns, while the remaining papers demonstrated a high risk of bias (Fig. 2).[28,32]

The Newcastle–Ottawa scale was used to assess the quality of evidence in 3 studies.[41–43] The analysis revealed that all studies had a good quality of evidence (Table 3).

Results of individual studies

In their study, Landes et al. assessed the effects of skeletal and dental expansion in the first and second molar and premolar regions after SARPE with BB or TB expanders.[32] The authors measured pre- and post-expansion on cone-beam computed tomography (CBCT). Additionally, the researchers assessed buccal and lingual vestibular bone resorption in the first premolar and first molar regions. The BB group comprised 24 participants, while the TB group contained 26 subjects. A comparison revealed a statistically significant difference in the skeletal expansion and buccal vestibular bone resorption in the first premolar region between the BB and TB groups. The BB group demonstrated a higher degree of skeletal expansion. However, the TB group exhibited greater vestibular bone resorption.[32]

Three of the included studies conducted their analyses on 3D cast models.[39,41,43] These studies assessed dental expansion in the canine, first premolar and first molar regions. Koudstaal et al. found a statistically significant difference in skeletal expansion in the first molar region, exhibiting increased expansion in the BB group.[39] Barone et al. observed a statistically significant difference in dental expansion in the first molar region, showing greater expansion in the TB group.[43] All 3 studies exhibited insignificant differences for dental expansion in the canine region.

Kayalar et al. performed their studies on CBCT, dividing 20 subjects equally into BB and TB groups.[40] Along with the skeletal and dental expansion, they also evaluated periodontal changes in the first premolar and first molar regions in subjects who underwent SARPE with BB or TB appliances. Furthermore, the post-expansion root length changes were assessed in conjunction with the intermolar and interpremolar angulations. The measurements were taken at 3 different time intervals: at baseline; after the expansion; and 6 months after the retention phase of expansion. A comparison of post-expansion changes between the BB and TB groups revealed significant differences in anterior dental expansion, first molar dental angulation, buccal and lingual alveolar bone thickness, and tooth

Table 1. Assessment of the quality of the evidence using the GRADE (Grading of Recommendations Assessment, Development and Evaluation) approach

Studies, *n*	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Summary of findings			
							patients, *n*		relative effect	certainty of evidence
							BB	TB		
5	RCT/non-RCT	not serious	not serious	not serious	not serious	none	86/170 (50.6%)	84/170 (49.4%)	not estimable	⊕⊕⊕⊕ high
2	cohort studies	not serious	not serious	not serious	not serious	none	35/79 (44.3%)	44/79 (55.7%)	not estimable	⊕⊕◯◯ low

RCT – randomized control trial; BB – bone-borne; TB – tooth-borne; *CI* – confidence interval.

Table 2. Characteristics of studies included in the review

Study	Study design	Sample size	Surgical procedure	Expander appliance	Primary outcome	Secondary outcome	Outcome assessment
Landes et al. 2009[32]	RCT	total: 50 BB: 24 TB: 26	bipartite median or tripartite paramedian osteotomy	BB: TPD and MWD TB: HE	skeletal and dental expansion in the first and second premolar regions, and in the first and second molar regions	dental tipping, vestibular bone resorption	CBCT
Koudstaal et al. 2009[39]	RCT	total: 46 BB: 25 TB: 21	Le Fort I with midline osteotomy	BB: TPD and BAD TB: HE	skeletal and dental expansion in the first premolar and first molar regions, dental expansion in the canine region	dental tipping	3D scanned cast models
Laudemann et al. 2010[41]	cohort study	total: 34 BB: 18 TB: 16	Le Fort I with midline osteotomy	BB: TPD and MWD TB: HE	dental expansion in the canine, first premolar and first molar regions	dental tipping, attachment loss	3D scanned cast models
Nada et al. 2012[42]	cohort study	total: 45 BB: 17 TB: 28	Le Fort I with midline osteotomy	BB: TPD TB: HE	skeletal and dental expansion in the first premolar and first molar regions, dental expansion in the canine region	none	CBCT
Zandi et al. 2014[28]	RCT	total: 30 BB: 15 TB: 15	Le Fort I with midline osteotomy	BB: TPD TB: HE	skeletal and dental expansion in the first premolar and first molar regions, nasal floor width	none	CBCT
Kayalar et al. 2016[40]	RCT	total: 20 BB: 10 TB: 10	Le Fort I with midline osteotomy	BB: hybrid RME TB: HE	skeletal and dental expansion in the first premolar and first molar regions	dental tipping, root resorption, vestibular bone resorption	CBCT
Barone et al. 2020[43]	non-RCT	total: 24 BB: 12 TB: 12	Le Fort I with midline osteotomy	BB: BAD TB: HE	dental expansion in the canine, first premolar and first molar regions	none	3D scanned cast models

TPD – transpalatal distractor; MWD – maxillary widening device; HE – Hyrax expander; BAD – bone-anchored device; RME – rapid maxillary expander; CBCT – cone-beam computed tomography.

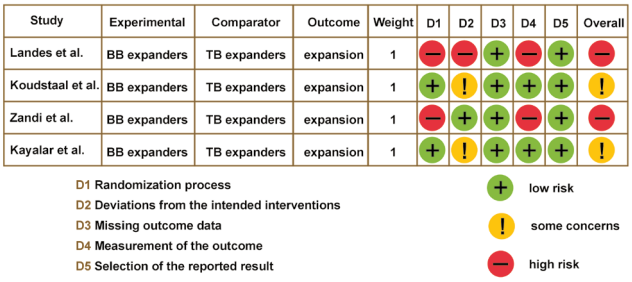

Fig. 2. Assessment of the risk of bias using the Cochrane's risk of bias (RoB 2.0) assessment tool for randomized controlled trials

BB – bone-borne; TB – tooth-borne.

Table 3. Assessment of the risk of bias using the Newcastle–Ottawa scale for non-randomized controlled trials and cohort studies

Study	Sample size	Selection	Comparability	Outcome
Laudemann et al.[41]	34	★★★	★	★★★
Nada et al.[42]	45	★★★	★	★★★
Barone et al.[43]	24	★★★	★	★★

good quality – 3 or 4 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain; fair quality – 2 stars in selection domain AND 1 or 2 stars in comparability domain AND 2 or 3 stars in outcome/exposure domain; poor quality – 0 or 1 star in selection domain OR 0 stars in comparability domain OR 0 or 1 stars in outcome/exposure domain.

length of the first premolars. A statistically significant difference in dental expansion within the first premolar region was identified, demonstrating greater expansion in the TB group. However, posterior dental expansion in the first molar region was comparable in both groups.

Zandi et al.[28] and Nada et al.[42] measured the dental and skeletal expansion in the first premolar and first molar regions in 30 and 45 participants, respectively. All participants underwent SARPE with BB or TB expanders. The latter also evaluated dental expansion in the canine region and found a statistically significant difference in this parameter between the 2 groups. Dental and skeletal expansion in other regions were comparable in both groups.

Meta-analysis

Five studies[28,32,39,40,42] were included in the meta-analysis to measure the extent of skeletal expansion in the first premolar and first molar regions of the BB and TB groups who underwent SARPE. Due to significant heterogeneity, the random-effects model was used for the synthesis of data from the first premolar region. No significant differences were observed (mean difference: 0.25; 95% confidence interval (*CI*): –0.27, 0.76) (Fig. 3). For the analysis of data in the first molar region, a fixed-effects model was employed. However, no statistically significant differences were observed between the groups (mean difference: 0.16; 95% *CI*: –0.34, 0.67) (Fig. 4).

The meta-analysis was conducted on a total of 7 studies, with the objective of measuring dental expansion in the first premolar and first molar regions in the BB and TB groups. Given the presence of significant heterogeneity among the studies, the random-effects model was used for the synthesis of data from the first premolar

region. The analysis revealed an absence of statistically significant differences (mean difference: –0.67; 95% *CI*: –1.45, 0.11) (Fig. 5). A fixed-effects model was used for the analysis of data from the first molar region. No statistically significant differences were observed between the 2 groups (mean difference: –0.29; 95% *CI*: –0.77, 0.19) (Fig. 6).

Four studies[32,39,42,43] were included in the meta-analysis to measure dental expansion in the canine region in the BB and TB groups. Due to significant heterogeneity in the data, the synthesis was performed using a random-effects model. The analysis revealed no statistically significant differences between the groups (mean difference: 0.05; 95% *CI*: –0.50, 0.60) (Fig. 7).

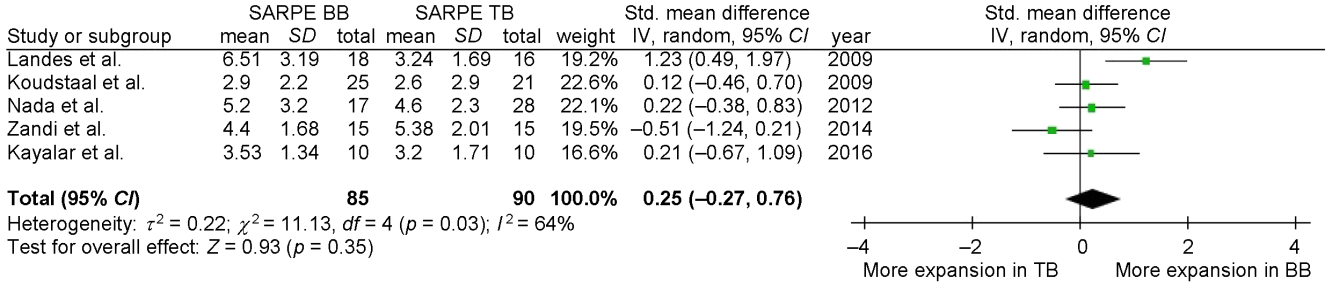

Fig. 3. Forest plot depicting the mean difference in skeletal expansion in the first premolar region between bone-borne (BB) and tooth-borne (TB) expanders after surgically assisted rapid palatal expansion (SARPE)

SD – standard deviation; *CI* – confidence interval; *df* – degrees of freedom.

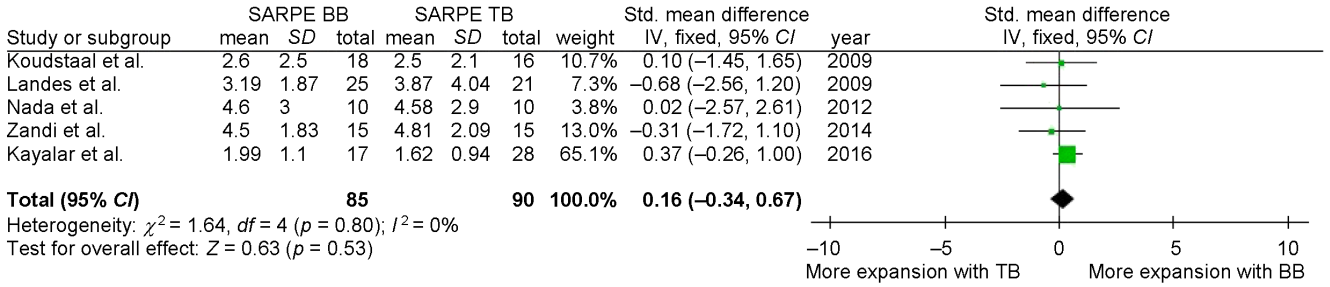

Fig. 4. Forest plot depicting the mean difference in skeletal expansion in the first molar region between BB and TB expanders after SARPE

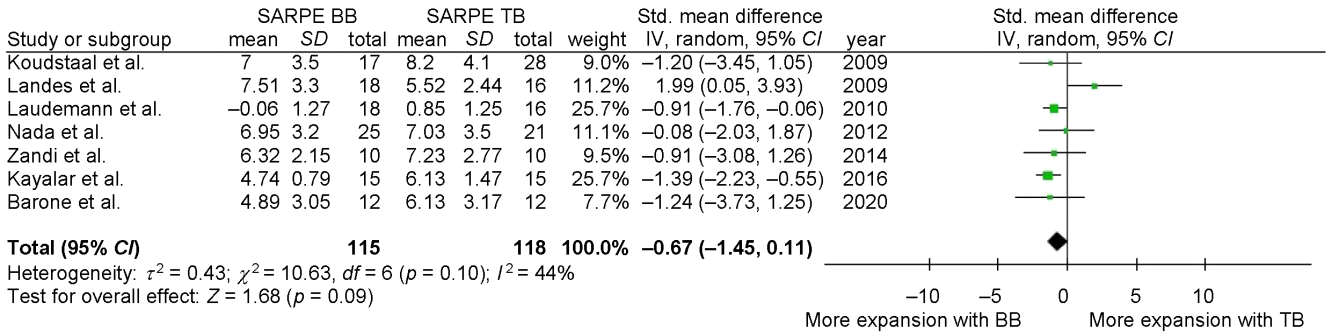

Fig. 5. Forest plot depicting the mean difference in dental expansion in the first premolar region between BB and TB expanders after SARPE

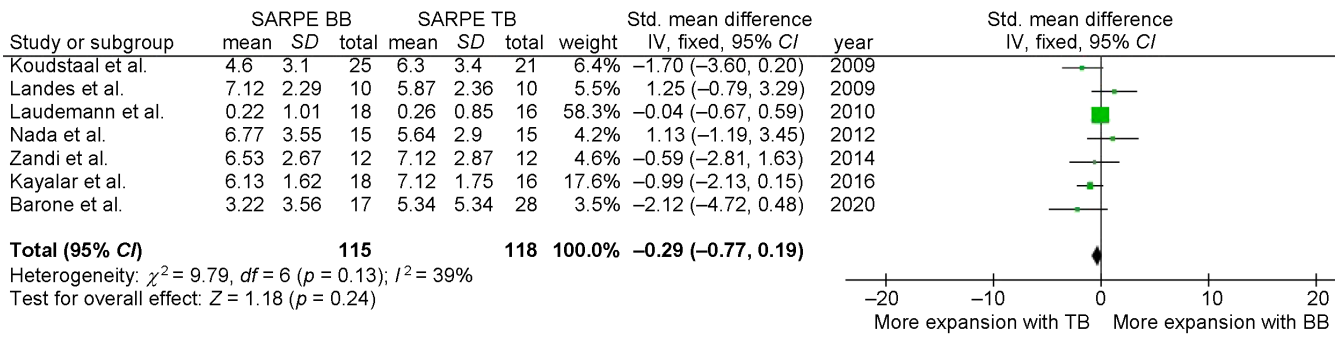

Fig. 6. Forest plot depicting the mean difference in dental expansion in the first molar region between BB and TB expanders after SARPE

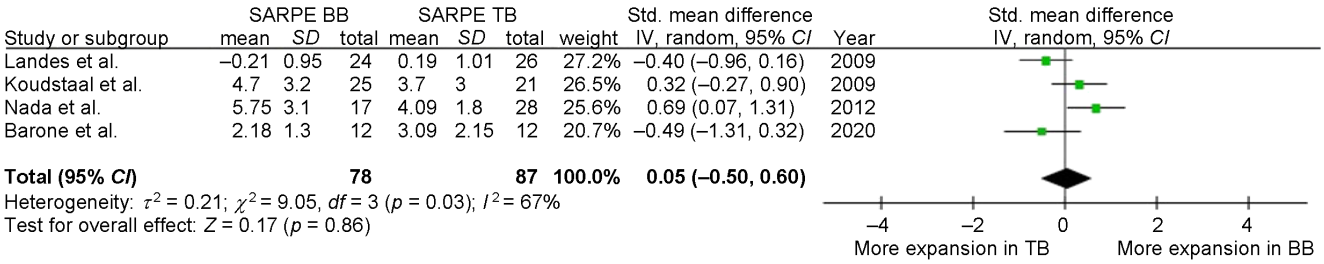

Fig. 7. Forest plot depicting the mean difference in dental expansion in the canine region between BB and TB expanders after SARPE

Assessment of the certainty of the evidence

The GRADE tool was employed to assess the findings from the included studies. The results demonstrated no differences in the expansion between the 2 techniques. The evaluation of the certainty of the evidence revealed that the clinical trials were of high quality, while the cohort studies were of low quality. Therefore, the significance of the findings of this review should be interpreted with caution (Table 1).

Discussion

This systematic review and meta-analysis compared skeletal and dental changes after BB and TB SARPE in subjects with MTD. The results showed that the dental and skeletal parameters in the transverse dimension were comparable between the 2 treatment modalities. The results hold strong evidence as only studies employing CBCT were considered. The clinical significance of the evidence obtained from this systematic review is highlighted by the GRADE scoring of all the clinical trials as high-quality strong evidence, while cohort studies provided low-quality evidence. To create strong evidence for skeletal expansion in the premolar and molar regions, we quantitatively analyzed the findings of 5 clinical trials by meta-analysis.[28,32,39,40,42] The Newcastle–Ottawa scale revealed that a non-RCT and cohort studies included in this systematic review and meta-analysis had good quality evidence.

In the adolescent patient population, maxillary expansion can be achieved through the use of TB appliances, either by slow or rapid expansion. In these patients, sutures are not yet fully fused, and the zygomaticomaxillary complex is in the process of development.[44] However, in adults, the fusion of intermaxillary and circummaxillary sutures is complete. Tooth-borne maxillary expansion after the maturation of these sutures mainly results in dentoalveolar effects rather than orthopedic effects. Thus, in adults, stable maxillary expansion can only be achieved through surgical interventions, such as maxillary osteotomy or SARPE. Both techniques involve a horizontal osteotomy of the lateral wall of the maxilla, with the separation of the lateral nasal wall, disarticulation of the nasal septum, and a palatal osteotomy. However, the maxilla is downfractured in a multiple-piece maxillary osteotomy, whereas SARPE does not involve this downward repositioning. Surgically-assisted rapid palatal expansion is a less complex and more physiological approach, and it provides a greater range of expansion due to tissue regeneration.[45] Although maxillofacial surgeons perform the surgery, the range of expansion is guided by an orthodontist with the help of surgical guides. Novel methods have been introduced to enhance the accuracy of these surgical guides, such as the use of 3D-printed resins like BioMed Amber.[46]

In order to create strong evidence for skeletal expansion in the premolar and molar regions, a quantitative analysis was performed on the findings of 5 clinical trials through meta-analysis.[28,32,39,40,42] Among all the included studies, only Landes et al. claimed that skeletal expansion is more pronounced in interpremolar width with BB transpalatal expander and maxillary widening device as compared to the TB Hyrax expander.[32] In this study, the surgical technique could be the primary factor contributing to significant skeletal expansion. The osteotomy technique employed in this study was either a bipartite median approach between the central incisor and bilaterally along the nasal septum or a tripartite paramedian approach between the lateral incisors and canines.[32] In the remaining 4 clinical trials, which demonstrated insignificant skeletal improvement in inter-premolar width, SARPE was performed as three-piece Le Fort I with midsagittal suture osteotomy. In all the studies, the appliance used for TB expansion was the Hyrax expander.

It has been well-documented that not only the midpalatal suture but also multiple sutures of the maxilla can contribute to arch constriction.[6,7,25] Interestingly, a significant skeletal intermolar expansion was observed in the findings of Nada et al., who reported greater skeletal expansion with transpalatal distractor as compared to the Hyrax appliance.[42] The analysis of dental expansion after SARPE involved assessing the tipping of the molars and premolars in all the included studies. All 7 studies included in this systematic review assessed dental expansion in the first premolar and first molar regions. The study by Kayalar et al. demonstrated that the TB Hyrax expander resulted in greater premoral tipping compared to the hybrid maxillary expander.[40] Surprisingly, increased molar tipping after SARPE was found in the

study by Barone et al.[43] These findings can be explained by the use of tooth-anchored appliances in these studies. Additionally, the buccal equilibrium resisted the skeletal suture opening of the maxilla, which requires heavy forces. Hence, it was concluded that an appliance attached to a tooth resulted in buccal tipping of molars before the skeletal opening of the suture.

Limitations

Among the included studies, Nada et al. assessed long-term treatment effects, with an analysis conducted 6 months after expander removal.[42] However, Landes et al. measured short-term postoperative effects and expansion after the treatment with TB and BB appliances.[32] The difference in follow-up duration could have influenced the findings. However, a meta-analysis was performed to eliminate these confounding factors. The majority of literature on the comparison of BB and TB expansion after SARPE are cross-sectional studies or literature book reviews. A comprehensive literature search yielded 5 clinical trials and 2 cohort studies that assessed these changes in longitudinal form. However, these studies had small sample sizes, which is the major limitation of this systematic review. The strength of this study lies in its exclusive inclusion of studies that employed CBCT as the assessment criterion, which is regarded as the gold standard.

To establish whether there is a difference between BB and TB SARPE, randomized clinical trials with larger sample sizes and longer follow-up periods should be conducted.

Conclusions

Within the limitations of insufficient evidence, this systematic review and meta-analysis concluded that there was no difference in skeletal and dental expansion in SARPE with BB and TB appliances. Due to the scarcity of available data, further studies are required to definitively ascertain clinical benefits of one treatment over another.

Trial registration

The current systematic review protocol has been registered with PROSPERO (CRD42022371097).

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.

ORCID iDs

Leelan Kanwal https://orcid.org/0000-0002-7916-0538
Hafsa Qabool https://orcid.org/0000-0002-8968-2014
Wafa Idrees https://orcid.org/0000-0002-5680-3430
Rashna Hoshang Sukhia https://orcid.org/0000-0001-9210-6432
Mubassar Fida https://orcid.org/0000-0003-4842-9896

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Review

Effect of tooth bleaching in patients with fixed orthodontic appliances: A systematic review

Gustavo Teodoro Costa Lizarelli[A–F], Rafaela Manente[B–F], Aline Souza-Gabriel[D–F], Silmara Aparecida Milori Corona[A,C,E,F]

Department of Restorative Dentistry, Ribeirão Preto School of Dentistry, University of São Paulo, Brazil

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):343–350

Address for correspondence
Silmara Aparecida Milori Corona
E-mail: silmaracorona@forp.usp.br

Funding sources
This study was funded by the National Council for Scientific and Technological Development (CNPq) (No. 160339/2021-9).

Conflict of interest
None declared

Acknowledgements
None declared

Received on July 13, 2023
Reviewed on July 27, 2023
Accepted on August 28, 2023

Published online on April 4, 2025

Abstract

Patients seeking a harmonious smile that combines satisfactory aesthetics and stable occlusion raise questions about the concomitant association of treatments. This systematic review aimed to answer the following question: "Does the application of hydrogen peroxide on teeth with fixed metallic orthodontic brackets interfere with the dental bleaching effect?". The PICOS (Population, Intervention, Comparison, Outcome, and Study design) strategy and the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines were adhered to during the review process. A comprehensive search strategy was implemented, encompassing the PubMed®, Web of Science, Scopus, Embase, and LILACS databases. The risk of bias was assessed using the Cochrane risk of bias (RoB) 2.0 tool. The initial search yielded 51 articles, which were then filtered to remove duplicates. The titles and abstracts of 26 studies were subsequently reviewed. Eleven articles were selected for full-text reading, and after applying the eligibility criteria, 3 studies were included for qualitative analysis. Two studies were classified as having a low risk of bias, while 1 study was classified as having some concerns. The bleaching was satisfactory with 8% and 10% hydrogen peroxide (HP) applied for 45 min daily over 10 days, even with fixed orthodontic brackets. However, a single study that employed the in-office technique (38% HP) demonstrated that fixed orthodontic appliances influenced external tooth bleaching. Tooth bleaching using the home bleaching technique yielded effective results when performed alongside orthodontic treatment.

Keywords: orthodontic brackets, index of orthodontic treatment need, tooth bleaching, tooth whitening

Cite as
Lizarelli GTC, Manente R, Souza-Gabriel A, Corona SAM. Effect of tooth bleaching in patients with fixed orthodontic appliances: A systematic review. *Dent Med Probl.* 2025;62(2):343–350. doi:10.17219/dmp/171606

DOI
10.17219/dmp/171606

Highlights

- The systematic review assessed the impact of hydrogen peroxide on dental bleaching for teeth with fixed metallic orthodontic brackets.
- Effective bleaching was achieved using 8–10% hydrogen peroxide for 45 min daily over 10 days, even with fixed orthodontic brackets.
- Tooth bleaching combined with orthodontic treatment improves both functional stability and smile aesthetics.

Introduction

Aesthetic procedures aimed at tooth whitening are commonly sought after by patients.[1–3] These procedures have been shown to have a significant psychosocial impact, affecting aesthetic self-perception.[4–8] Tooth bleaching is one of the most commonly performed procedures in the dental office, especially in cases of tooth discoloration, tetracycline stains and extrinsic stains combined with the patient's diet.[9–11] It is a conservative alternative for restoring aesthetics in vital and non-vital teeth.[12]

The mechanism of action of the bleaching gel involves diffusion[9,13,14] due to the characteristic permeability of dental tissues (enamel and dentin) associated with low molecular weight and the ability of hydrogen peroxide (HP) to generate free radicals.[15,16] Diffusion capacity is related to factors such as the patient's age, which is a determining factor for the deposition of secondary dentin throughout life, causing the thickness of this dentin to increase and, consequently, decreasing the efficiency and penetration of HP.[9,17] Moreover, the diffusion capacity is associated with the concentration of peroxide,[18,19] application time, amplitude of dentinal tubules,[20] and variation in dental structure.[21]

Tooth bleaching is a minimally invasive approach that provides excellent aesthetic results.[22,23] Its applications have expanded in the field of orthodontics. Orthodontic treatment can result in changes to tooth color, prompting many patients to seek alternatives for restoring their tooth color.[24] Tooth bleaching has been shown to enhance the dental substrate, thereby enabling aesthetic procedures to re-establish the shape and size of teeth. This includes closure of the diastema and re-anatomization of conoid teeth, which are not fully addressed by orthodontics.[25,26]

The most common method of clinically assessing tooth color is through visual shade matching. This approach is quick and simple to apply. However, the subjective nature of this method stemming from variations in the perception of colors by the observer, lighting conditions, translucency, and the optical properties of the material examined, may affect the results.[27,28] Another method of assessing tooth color is through the use of a spectrophotometer, which was invented in 1940 by Beckman et al.[29] This tool measures the reflection properties of an object and converts them into color coordinates and various tooth shade values.

The use of HP in tooth bleaching procedures results in a polydirectional whitening effect, encompassing areas covered by orthodontic brackets.[30] Additionally, it helps control plaque and reduce gingivitis or periodontal diseases during treatment. Furthermore, it enables patients to achieve whitened teeth prior to orthodontic finishing, thereby optimizing time management.[31] A survey conducted among US orthodontic specialists revealed that a high percentage of participants (88.8%) reported that their patients requested tooth bleaching.[32] Another study demonstrated that patients exhibited higher levels of satisfaction with orthodontic treatment when it was combined with bleaching.[33] Hydrogen peroxide assists in regulating oral biofilm, and the bleaching procedure on teeth with brackets potentially serves as a motivational factor, preventing treatment withdrawal and reducing the total treatment duration.[31]

Clinicians could potentially consider a combination of both treatments (bleaching and orthodontics) to establish a stable functional occlusion and harmonious dentofacial aesthetics. Therefore, based on the scientific literature on the characteristics and advantages of tooth bleaching and its association with orthodontic treatment, the purpose of this systematic review was to ascertain whether tooth bleaching gel can be effective on tooth surfaces and whether it can provide satisfactory aesthetic outcomes in the presence of orthodontic brackets.

Material and methods

Protocol

This systematic review was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines based on the PICOS (Population, Intervention, Comparison, Outcome, and Study design) strategy, as follows: population (P) – patients with fixed orthodontic brackets; intervention (I) – tooth bleaching; comparison (C) – individuals with and without brackets; outcome (O) – bleaching effect; study design (S) – in vivo research articles. The review aimed to answer the following question: "Does the application of hydrogen peroxide on teeth with fixed metallic orthodontic brackets interfere with the dental bleaching

effect?". The study was registered with PROSPERO (registration No. CRD42023408848).

Search strategy

The literature search was independently conducted by 2 researchers (GTCL and RM) for reports published until May 2023 in the Embase, PubMed®, Scopus, Web of Science, and LILACS databases. English terms from Medical Subject Headings (MeSH) and free keywords with Boolean operators (OR, AND) were used in the search. The search strategy was adapted to each database and is listed in Table 1.

Eligibility criteria

The present review included clinical studies that evaluated patients who underwent tooth bleaching with HP in varying concentrations and with different application protocols. Studies that did not meet the eligibility criteria, as well as systematic reviews, editorials, letters, case reports, and in vitro studies that aligned with the proposed research question were excluded from the analysis.

Selection process

The articles were evaluated independently by 2 reviewers (GTCL and RM) in 2 stages according to the eligibility criteria. In the first phase, the selection of studies was based on the analysis of titles and abstracts, and in the second phase, the full-text articles were examined. Any doubts and discrepancies were addressed through consensus meetings with the coordinator (SAMC). Initially, the authors conducted a search of relevant databases and excluded duplicate and irrelevant articles. The titles and abstracts were then reviewed, and those that did not address the review question were removed. The remaining studies were read in full, and those that did not match the subject under investigation were excluded. Finally, articles meeting all the selection criteria were included (Fig. 1).

Each study was assigned an ID based on the name of the first author and the year of publication. Extraction forms customized for the study were used to gather information on study methods, designs and settings, characteristics of the participants, and bleaching protocols.

Risk of bias in individual studies

The risk of bias was assessed by 2 reviewers using the Cochrane risk of bias (RoB) 2.0 tool. This tool contains several items, including allocation concealment, sequence generation, blinding of participants and outcome assessors, incomplete outcome data, and selective outcome reporting. The evaluation of each domain of the RoB tool was conducted in accordance with the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions, 5.1.0.[34] The assessments of low risk, high risk, or some concerns were made based on the presence or absence of information or uncertainty regarding the potential for bias.

Table 1. Search strategies for each database

Database	Strategy
PubMed®	(tooth bleaching [MeSH Terms]) OR (teeth whitening [MeSH Terms]) OR (teeth bleaching [MeSH Terms]) OR (carbamide [MeSH Terms]) OR (hydrogen peroxide [MeSH Terms]) OR (agents, teeth whitening [MeSH Terms]) OR (agents, tooth whitening [MeSH Terms]) OR (teeth whitening agents [MeSH Terms]) OR (agents, bleaching [MeSH Terms]) AND (brackets, orthodontic [MeSH Terms]) OR (bracket, orthodontic [MeSH Terms]) OR (orthodontic bracket [MeSH Terms]) OR (orthodontic brackets [MeSH Terms]) AND (index of orthodontic treatment need [MeSH Terms]) OR (index of orthodontic treatment needs [MeSH Terms]) OR (index for need of orthodontic treatment [MeSH Terms])
Embase	(tooth bleaching: ti,ab,kw OR teeth whitening: ti,ab,kw OR teeth bleaching: ti,ab,kw OR carbamide: ti,ab,kw OR hydrogen peroxide: ti,ab,kw OR agents, teeth whitening: ti,ab,kw OR agents, tooth whitening: ti,ab,kw OR teeth whitening agents: ti,ab,kw OR agents, bleaching AND brackets, orthodontic: ti,ab,kw OR bracket, orthodontic: ti,ab,kw OR orthodontic bracket: ti,ab,kw OR orthodontic brackets: ti,ab,kw AND index of orthodontic treatment need: ti,ab,kw OR (index of orthodontic treatment needs: ti,ab,kw OR index for need of orthodontic treatment))
LILACS	(mh: (tooth bleaching)) OR (mh: (teeth whitening)) OR (mh: (teeth bleaching)) OR (mh: (carbamide)) OR (mh: (hydrogen peroxide)) OR (mh: (agents, teeth whitening)) OR (mh: (agents, tooth whitening)) OR (mh: (teeth whitening agents)) OR (mh: (agents, bleaching)) AND (tw: (brackets, orthodontic)) OR (tw: (bracket, orthodontic)) OR (tw: (orthodontic bracket)) OR (tw: (orthodontic brackets)) AND (tw: (index of orthodontic treatment need)) OR (tw: (index of orthodontic treatment needs)) OR (tw: (index for need of orthodontic treatment))
Web of Science	topic: tooth bleaching OR teeth whitening OR teeth bleaching OR carbamide OR hydrogen peroxide OR agents, teeth whitening OR agents, tooth whitening OR teeth whitening agents OR agents, bleaching AND brackets, orthodontic OR bracket, orthodontic OR orthodontic bracket OR orthodontic brackets AND index of orthodontic treatment need OR index of orthodontic treatment needs OR index for need of orthodontic treatment
Scopus	(Title-ABS-KEY: tooth bleaching OR Title-ABS-KEY: teeth whitening OR Title-ABS-KEY: teeth bleaching OR Title-ABS-KEY: carbamide OR Title-ABS-KEY: hydrogen peroxide OR Title-ABS-KEY: agents, teeth whitening OR Title-ABS-KEY: agents, tooth whitening OR Title-ABS-KEY: teeth whitening agents OR Title-ABS-KEY: agents, bleaching AND Title-ABS-KEY: brackets, orthodontic OR Title-ABS-KEY: bracket, orthodontic OR Title-ABS-KEY: orthodontic bracket OR Title-ABS-KEY: orthodontic brackets AND Title-ABS-KEY: index of orthodontic treatment need OR Title-ABS-KEY: index of orthodontic treatment needs OR Title-ABS-KEY: index for need of orthodontic treatment)

MeSH – Medical Subject Headings; ti – searches for the presence of a word (keyword) in the title of an article; ab – searches for the presence of a word (keyword) in the abstract of an article; kw – searches for author-provided keywords; mh – MeSH terms; tw – Text Word (searches for specific words or phrases in the text of an article); ABS – abstract; KEY – keywords.

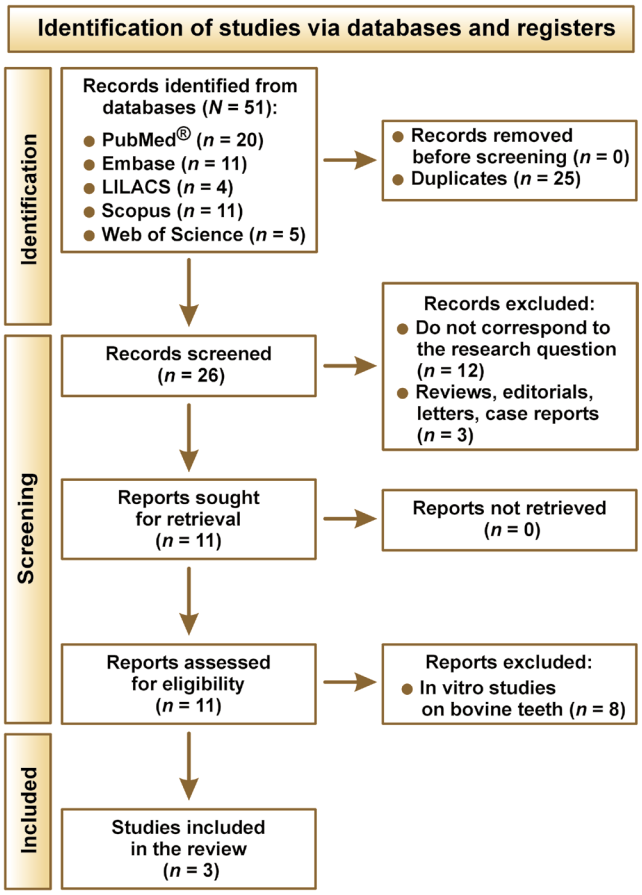

Fig. 1. Flowchart of the study

Results

Study selection

During the search, a total of 51 articles published until May 2023 were retrieved, and a pre-selection was performed after the removal of duplicates, yielding 26 articles. Eleven potentially eligible articles were selected for further evaluation after a review of their titles and abstracts. Subsequent to a thorough examination of the texts, 8 articles were excluded because they were not clinical studies, leaving 3 articles that were eligible for the present review.

Characteristics of the included articles

The following information was retrieved from each study: author/year of publication; number and age of the participants; groups; bleaching protocol; response variables; and results (Table 2). The 3 articles included in this review[35–37] were comparative clinical studies performed using parity between groups. Tooth staining was evaluated after tooth bleaching in patients undergoing orthodontic treatment with fixed brackets. The patients had a similar mean age.

For the purpose of color analysis, the studies used a digital system that utilized a spectrophotometer. The instrument enables the representation of colors in the International Commission on Illumination (CIE) L*a*b* format. Two studies used the VITA Easyshade® spectrophotometer (Vident, Brea, USA),[35,37] and 1 study used the SpectroShade Micro spectrophotometer (MHT, Zurich,

Table 2. Characteristics of studies included in the review

Study	Participants, *n*	Groups	Age [years]	Bleaching protocol	Response variables	Results
Jadad et al. 2011[35]	40	– bleaching with a fixed orthodontic bracket – bleaching without a fixed orthodontic bracket	18–40	8% HP – 45 min/day for 10 days	color: VITA Easyshade® spectrophotometer (Vident, Brea, USA)	Bleaching was effective in both groups.
Montenegro-Arana et al. 2016[37]	40	– bleaching with an orthodontic bracket (Trèswhite Ortho bleaching gel) – bleaching with an orthodontic bracket (Trèswhite Supreme bleaching gel)	18–40	– Trèswhite Ortho group: 8% HP – 45 min/day for 10 days – Trèswhite Supreme group: 10% HP – 45 min/day for 10 days	color: VITA Easyshade® spectrophotometer (Vident) sensitivity: 5-point scale, numerical rating scale ranging from 0 to 100, VAS	Both bleaching gels were effective.
Koumpia et al. 2022[36]	72	– debonded – undergoing retention – untreated	18–58	38% HP – according to the manufacturer's instructions	color: SpectroShade Micro reflectance spectrophotometer (MHT, Zurich, Switzerland), CIE L*a*b* system	Fixed orthodontic appliances influenced the bleaching efficacy. Tooth bleaching treatment had a greater effect when administered after orthodontic treatment.

HP – hydrogen peroxide; VAS – visual analog scale; CIE – International Commission on Illumination; L* – lightness; a* – red/green coordinate; b* – yellow/blue coordinate.

Switzerland) and the CIE L*a*b* format to calculate L* (lightness ranging from 0 (black) to 100 (white)), a* (red/green coordinate) and b* (yellow/blue coordinate) parameters.[36] Additionally, 1 study evaluated tooth sensitivity using a visual analog scale (VAS), a 5-point scale and a numerical rating scale ranging from 0 to 100.[37]

The number of patients included in the studies was 40,[35] 72[36] and 40.[37] In the 3 studies, the minimum age of the participants was 18 years, and the age range was 18–40 years,[35] 18–58 years,[36] and 18–40 years.[37] Notably, 1 study documented a higher proportion of female participants compared to male participants,[36] while the other 2 studies did not provide specific demographic data.

One of the 3 studies assessed tooth sensitivity.[37] The patients from 2 groups (Trèswhite Ortho and Trèswhite Supreme), both undergoing orthodontic treatment, were instructed to report the level of pain during the 10 bleaching sessions using VAS ranging from 0 to 4 (0 = none, 1 = mild, 2 = moderate, 3 = considerable, and 4 = severe) and a numeric rating scale ranging from 0 to 100. The highest values recorded for each session were divided into 2 categories: absolute risk of tooth sensitivity; and overall intensity of tooth sensitivity. The analysis revealed no statistically significant differences for both categories, with *p*-values of 0.53 and >0.05, respectively, for absolute risk of tooth sensitivity and overall intensity of tooth sensitivity.[37]

With regard to the bleaching protocol, the most frequently used agent was HP gel, and its concentration ranged from 8% to 38%. The gel application protocol varied among the studies: 8% HP was used for 45 min/day over 10 days[35]; 8% and 10% HP were used for 45 min/day over 10 days[37]; and 38% HP was used according to the manufacturer's instructions.[36]

A comparative analysis of the articles revealed similarities between the groups of teeth subjected to bleaching. Jadad et al.,[35] Koumpia et al.[36] and Montenegro-Arana et al.[37] included 6 maxillary anterior teeth in their studies. The outcomes of each study indicated that the bleaching protocols generally yielded positive results,[35,37] with the exception of 1 study,[36] the authors of which concluded that tooth bleaching treatment had a greater effect when administered after orthodontic treatment (Table 2).

Risk of bias

In accordance with the RoB 2.0 tool, the studies by Montenegro-Arana et al.[37] and Koumpia et al.[36] were rated "low risk", while the study by Jadad et al.[35] was rated "with some concerns" (Fig. 2).

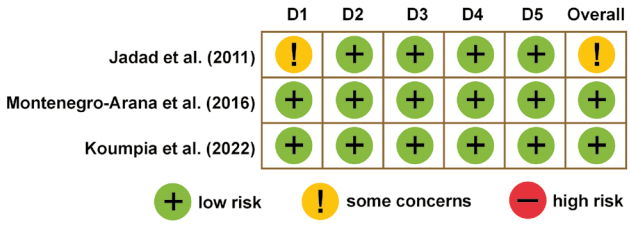

Fig. 2. Risk of bias in the randomized trials included in the review, assessed using the Cochrane risk of bias (RoB) 2.0 tool

D1 – bias arising from the randomization process; D2 – bias due to deviations from intended interventions; D3 – bias due to missing outcome data; D4 – bias in measurement of the outcome; D5 – bias in selection of the reported result.

Discussion

Bleaching is an aesthetic procedure that can be performed after the alignment of teeth and before the dental re-anatomization with composite resin.[38] When performed in conjunction with orthodontic therapy, it provides motivated patients with stable functional occlusion and harmonious aesthetics in a shorter period of time.[32,33,35,37] The existing evidence regarding the effect of bleaching on teeth that have undergone orthodontic treatment is inconclusive, thereby supporting the need for evidence-based information regarding such protocols. To the best of our knowledge, this is the first literature review to assess the outcomes of in vivo studies using concomitant tooth bleaching and orthodontic brackets.

Modifications in enamel color are associated with surface alterations due to irreversible penetration of resin tags following orthodontic bonding,[39] direct absorption of food colorants and products resulting from corrosion of the orthodontic appliance,[40] as well as decalcification and grooving during adhesive removal.[41] These disadvantages could potentially be avoided through tooth bleaching during orthodontic treatment. This hypothesis is based on the mechanism of action (diffusion) of bleaching gels under orthodontic brackets.[30] Hydrogen peroxide has a low molecular weight and the ability to generate free radicals, which diffuse easily into the lamellae, grooves, fissures, and depressions of the teeth.[15,16] Moreover, the variability in enamel pores, both in terms of size and location within or between hydroxyapatite prisms, has been demonstrated to affect treatment outcomes.[21] A previous systematic review evaluated the efficacy of home tooth bleaching combined with orthodontic aligners and showed positive outcomes with carbamide or HP.[42]

In this review, 2 studies demonstrated a favorable response to the home bleaching technique when combined with conventional orthodontic treatment.[35,37] Jadad et al. observed that the bleaching protocol of 8% HP (45 min/day for 10 days) simultaneous with orthodontic treatment provided satisfactory aesthetic results, similar to those obtained with post-orthodontic bleaching treatment.[35] Montenegro-Arana et al. used 2 bleaching agents (Trèswhite Ortho and Trèswhite Supreme) with a protocol involving the application of 8% and 10% HP (45 min/day) for 10 days, in 2 groups of patients with fixed orthodontic appliances.[37] The study yielded positive outcomes for tooth bleaching with both bleaching agents.[37]

However, the findings of Koumpia et al. differed from those of other studies.[36] The authors tested in-office bleaching with 38% HP, according to the manufacturer's instructions. They found that the orthodontic brackets had an influence on the bleaching effect. The bleaching process was associated with an increase in the L* value and a decrease in the a* and b* values in orthodontically-treated and untreated teeth. The color difference (ΔE), L* and b* parameters demonstrated significant differences between the groups not undergoing orthodontic treatment and those in the orthodontic retention phase. This outcome suggests that the bonding and debonding processes that took place during orthodontic treatment might have had a negative influence on the bleaching results.[36]

Debonding leads to morphological changes, such as the loss of enamel structure, which directly interferes with light reflection.[36] Additionally, debonding results in enamel discoloration due to the presence of residual adhesive within the prisms, as well as increased roughness and small fractures.[43–50] The discoloration of the adhesive affects the color of the enamel through physicochemical reactions and the absorption of food pigments.[45,51,52]

A plausible explanation for positive results observed with HP-based gels at low concentrations is that concentration and duration are key factors affecting the efficacy of bleaching treatments.[53] High concentrations demonstrate faster results than low concentrations. However, low concentrations can be equally effective if the treatment duration is extended. Additionally, the active agent in the bleaching gel only reaches the dentin region immediately below the enamel. This suggests that a low concentration and reduced penetrating power are necessary to achieve effective bleaching results.[54]

Because free radicals act in a polydirectional manner, they exert an effect under the brackets and resin adhesives, cementing orthodontic appliances.[55] The rheological properties of Opalescence Trèswhite Ortho gel are improved by the presence of polymers, which contributes to the control of oxygen release and the cohesion of the product on the tooth surface. In the context of orthodontic appliances, this effect maximizes the efficiency of the bleaching agent.[55]

The studies included in this review used either the VITA Easyshade® or the SpectroShade Micro spectrophotometer for color analysis. Both devices exhibited high accuracy[56,57] and reproducibility for tooth bleaching evaluation.[58] Montenegro-Arana et al. evaluated tooth color before and 30 days after the bleaching procedure using the VITA Easyshade® device.[37] The results were assigned values based on the VITA classical color scale. Similarly, Jadad et al.[35] used VITA Easyshade® and the same color allocation parameters as those used in the study by Montenegro-Arana et al.[37] Koumpia et al. utilized the SpectroShade Micro reflectance spectrophotometer, and the color standard was analyzed using the CIE L*a*b* system.[36]

The use of different methodologies and devices for measuring the color before and after bleaching, as well as the application of different protocols and concentrations of the bleaching gel, may have contributed to the divergence in the studies regarding home and in-office techniques.

Overall, this review has determined a change in tooth color to lighter shades, thereby confirming the efficacy of the bleaching gel in the presence of orthodontic brackets for the home bleaching technique. The limitations of this review are due to the low number of studies assessed. Additionally, one of the included studies did not incorporate a randomization process for the samples. Therefore, further randomized clinical studies on the combined treatment and with a longer follow-up are recommended to elucidate its effects on the tooth structure over time.

Conclusions

In light of the limitations inherent to a systematic review and based on the results of the selected studies, it can be concluded that the concomitant application of orthodontic brackets and tooth bleaching offers favorable outcomes for patients in terms of aesthetics and function. The procedure of tooth bleaching was effective when using protocols based on 8% and 10% HP for 45 min/day over a period of 10 days.

Trial registration

The study was registered with PROSPERO (registration No. CRD42023408848).

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.

ORCID iDs

Gustavo Teodoro Costa Lizarelli https://orcid.org/0000-0001-7805-0399
Rafaela Manente https://orcid.org/0000-0002-1740-6192
Aline Souza-Gabriel https://orcid.org/0000-0002-9280-2945
Silmara Aparecida Milori Corona https://orcid.org/0000-0002-1733-3472

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Review

Salivary cytokines as a biomarker for diagnosis, prognosis and treatment of oral squamous cell carcinoma: A systematic review

Gayathri Rengasamy[1,A–C], Hema Shree Kasirajan[2,A,B], Vishnu Priya Veeraraghavan[1,A–C], Pratibha Ramani[2,A–C], Gabriele Cervino[3,D–F], Giuseppe Minervini[4,5,D–F]

[1] Department of Biochemistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India
[2] Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India
[3] Department of Biomedical and Dental Sciences, Morphological and Functional Images, G. Martino Polyclinic, University of Messina, Italy
[4] Department of Orthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India
[5] Multidisciplinary Department of Medical-Surgical and Dental Specialties, University of Campania Luigi Vanvitelli, Naples, Italy

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):351–359

Address for correspondence
Giuseppe Minervini
E-mail: giuseppe.minervini@unicampania.it

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on February 29, 2024
Reviewed on March 25, 2024
Accepted on March 30, 2024

Published online on April 17, 2025

Abstract

Oropharyngeal squamous cell carcinoma and oral squamous cell carcinoma (OSCC) are the most common types of head and neck squamous cell carcinoma. Oral squamous cell carcinoma is the 6th most prevalent epithelial malignancy worldwide, and it is known for having significant morbidity and mortality rates. The present systematic review aims to identify which cytokines can be used as salivary biomarkers for the prognosis, diagnosis and treatment of OSCC. The early detection of the tumor and its precursor lesions is critical for improving the survival rate, reducing costs and enhancing the quality of life following treatment for OSCC. The conducted literature search yielded 65 articles; however, only 8 articles met the inclusion and exclusion criteria and were selected for a systematic review. Eighty percent of the articles were review articles, encompassing case–control studies and longitudinal studies. In 50% of the studies, diagnostic meta-analyses were conducted. According to the reviewed articles, interleukin-1β (*IL-1β*), *IL-6*, *IL-8*, *IL-1* receptor antagonist (*IL-1RA*), interferon-γ (*IFN-γ*), tumor necrosis factor (*TNF*) (*-α*, *-β*, *-γ*), and matrix metalloproteinase-9 (*MMP-9*) are potential markers for OSCC, with a sensitivity and specificity of 100%.

Keywords: salivary cytokines, biomarkers, oral squamous cell carcinoma

Cite as
Rengasamy G, Kasirajan HS, Veeraraghavan VP, Ramani P, Cervino G, Minervini G. Salivary cytokines as a biomarker for diagnosis, prognosis and treatment of oral squamous cell carcinoma: A systematic review. *Dent Med Probl.* 2025;62(2):351–359
doi:10.17219/dmp/186664

DOI
10.17219/dmp/186664

Highlights

- Salivary cytokines such as *IL-1β*, *IL-6*, *IL-8*, and *TNF*s serve as potential biomarkers for oral squamous cell carcinoma (OSCC).
- Saliva is a non-invasive, low-cost medium for OSCC detection and ongoing disease monitoring.
- Some studies demonstrate diagnostic accuracy of salivary cytokines with up to 100% sensitivity and specificity.
- Further large-scale, standardized clinical studies are required to confirm the diagnostic utility of salivary cytokines in OSCC.

Introduction

The mucosal epithelium lines the larynx, hypopharynx, oropharynx, nasopharynx, and oral cavity. It is in these regions that squamous cell carcinoma of the head and neck originates. Squamous cell carcinomas are the most prevalent histological manifestation of head and neck cancer, accounting for 90% of all head and neck malignancies.[1] Oropharyngeal squamous cell carcinoma and oral squamous cell carcinoma (OSCC) are the most common types of head and neck squamous cell carcinoma.[2] Oral squamous cell carcinoma is the 6th most prevalent epithelial malignancy worldwide, and is known for its significant morbidity and mortality rate. Consequently, growing worldwide public health concerns are related to OSCC.[2]

According to the World Health Organization (WHO), the five-year mortality rate for oral cancer is 45%.[3] There is an 80–90% chance of survival if oral cancer is discovered in its early stages.[4] Unfortunately, due to inadequate public education and screening techniques, these malignancies are difficult to detect in the early stages, which typically has a negative impact on prognosis and survival rate.[5] A reliable early diagnosis of the tumor and its initial lesions is required for a decrease in treatment costs, an increase in the survival rate, and an enhancement of the quality of life following OSCC treatment.[6]

Comprehensive clinical examinations, costly biochemical analyses and invasive biopsies remain the gold standard for detecting oral malignancies.[7–9] The discovery of biomarkers in biological matrices (blood, urine and saliva) may potentially help in the early identification of disease.[10] Saliva is a biofluid made up of components that can be utilized as biomarkers, including cytokines, RNA and DNA molecules, circulatory cells, and microvesicles.[11] In the last decade, research has focused on the examination of bodily fluids, also referred to as "liquid biopsy", to identify biomarkers that could be predictive or diagnostic in OSCC.[12] Notably, due to its accessibility, proximity to cancer cells non-invasiveness and cost-effectiveness, saliva has emerged as a promising biological specimen for early cancer detection.[13] Consequently, the use of saliva for early detection of cancer is a promising strategy in the search for new diagnostic and therapeutic biomarkers.[14–33]

Advances in the understanding of the molecular development of OSCC have facilitated the recognition of several potential biomarkers in unstimulated whole saliva, indicating changes in genomic and epigenetic processes, as well as metabolic or proteomic activity, in malignant cells.[11,34,35] This knowledge encourages the prompt identification and diagnosis of OSCC.[11] Cytokines are main intercellular protein mediators of the immune system.[36] They are essential for growth, development and healing in addition to regulating immunological processes.[37] Additionally, they facilitate communication between the body's immune system and other systems. Cytokines are synthesized and secreted by cells throughout the body and central nervous system (CNS). While the secretion of cytokines often occurs concurrently with protein synthesis, some are produced and retained for rapid release within intracellular granules. The effects of cytokines are frequently redundant or synergistic, and they have a variety of target cells and modes of action.

The production of certain cytokines by OSCC cells has been demonstrated. It has been proposed that these cytokines play a part in the formation of tumors and in the process of angiogenesis.[38] Moreover, these cytokines have been proven to be present in saliva. Consequently, salivary cytokines have the potential to serve as crucial biomarkers for the evaluation, prognosis and treatment of OSCC.

Material and methods

A total of 67 articles published between 2019 and 2024 were identified through manual searches of PubMed®, Scopus, LILACS, Google Scholar, Embase, and Cochrane Library databases. The inclusion criteria for this review were studies published between 2019 and 2024, which utilized salivary cytokines as biomarkers. The case–control studies, longitudinal studies, clinical trials, and reviews were included. Human studies alone were considered for the inclusion, whereas in vitro studies and animal studies were excluded. Two individual reviewers independently evaluated the articles for risk assessment based on the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) criteria. The results are

presented in Fig. 1 and Fig. 2. Following a thorough evaluation of the articles based on the predetermined inclusion criteria and risk assessment, 8 articles were selected for inclusion in this systematic review.

Data extraction

A comprehensive search of PubMed®, Scopus, LILACS, Google Scholar, Embase, and Cochrane Library databases was conducted using the following search terms: alveolar cancer; alveolar cancers; alveolar tumor; alveolar tumors; alveolar carcinoma; alveolar carcinomas; alveolar malignancy; alveolar malignancies; alveolar neoplasm; alveolar neoplasms; gingivobuccal cancer; gingivobuccal cancers; gingivobuccal tumor; gingivobuccal tumors; gingivobuccal carcinoma; gingivobuccal carcinomas; gingivobuccal malignancy; gingivobuccal malignancies; gingivobuccal neoplasm; gingivobuccal neoplasms; cheek cancer; cheek cancers; cheek tumor; cheek tumors; cheek carcinoma; cheek carcinomas; cheek malignancy; cheek malignancies; cheek neoplasm; cheek neoplasms; tongue cancer; tongue cancers; tongue tumor; tongue tumors; tongue carcinoma; tongue carcinomas; tongue neoplasm; tongue neoplasms; tongue malignancy; tongue malignancies; lingual cancer; lingual cancers; lingual tumor; lingual tumors; lingual carcinoma; lingual carcinomas; lingual malignancy; lingual malignancies; lingual neoplasm; lingual neoplasms; buccal cancer; buccal cancers; buccal tumor; buccal tumors; buccal carcinoma; buccal carcinomas; buccal malignancy; buccal malignancies; buccal neoplasm; buccal neoplasms; oral cancer; oral cancers; oral tumor; oral tumors; oral carcinoma; oral carcinomas; oral malignancy; oral malignancies; oral neoplasm; oral neoplasms; mouth cancer; mouth cancers; mouth tumor; mouth tumors; mouth carcinoma; mouth carcinomas; mouth malignancy; mouth malignancies; mouth neoplasm; mouth neoplasms; head and neck tumor; head and neck tumors; head and neck carcinoma; head and neck carcinomas; head and neck malignancy; head and neck malignancies; head and neck neoplasm; head and neck neoplasms.

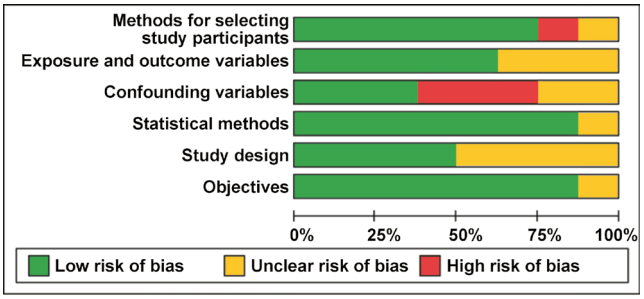

Fig. 1. Assessment of the risk of bias across all included studies based on the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) criteria

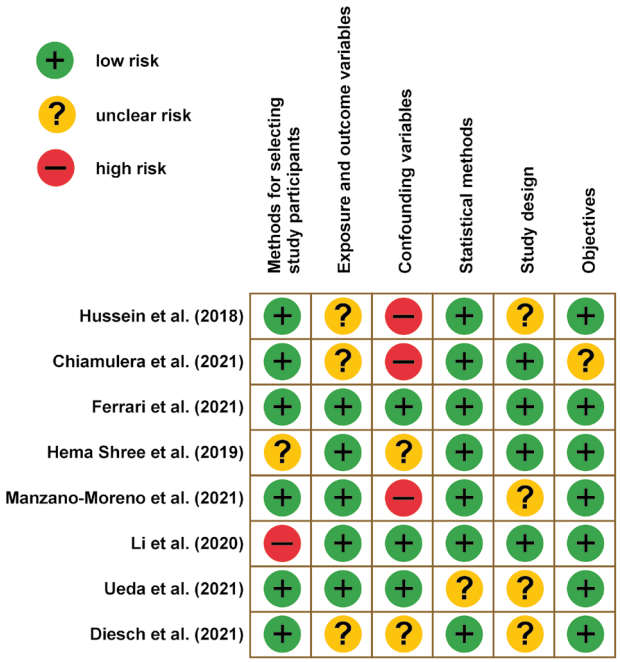

Fig. 2. Assessment of the risk of bias for each included study based on the STROBE criteria

Results

A comprehensive data search yielded 65 articles, of which 8 were selected for systematic review based on the inclusion and exclusion criteria. Eighty percent of the articles were review articles, encompassing case–control studies and longitudinal studies. Furthermore, 50% of the studies conducted diagnostic meta-analyses. In 80% of the articles, tumor necrosis factor (*TNF*) (*-α*, *-β*, *-γ*), matrix metalloproteinase-9 (*MMP-9*), interleukin-1β (*IL-1β*), *IL-6*, *IL-8*, interleukin-1 receptor antagonist (*IL-1RA*), and interferon-γ (*IFN-γ*) were discussed as potential markers for OSCC. The sensitivity and specificity of these markers were reported to be 100%.

Consequently, a panel of highly specific markers can be established for the detection of OSCC. These markers could also facilitate the analysis of prognosis and treatment outcomes for patients with OSCC.

Discussion

This systematic review underscores the significant potential of inflammatory cytokines and matrix-degrading enzymes as salivary biomarkers in the detection of OSCC. The consistent appearance of markers such as *TNF* variants, *MMP-9*, *IL-1β*, *IL-6*, *IL-8*, *IL-1RA*, and *IFN-γ* across the majority of studies highlights a growing consensus in the field. These biomarkers not only reflect underlying pathophysiological processes but also present an opportunity to develop non-invasive, highly accurate diagnostic tools.

Table 1. Characteristics of the included studies on the use of salivary cytokines as diagnostic, prognostic and treatment biomarkers in patients with oral squamous cell carcinoma (OSCC)

Study	Sample size, *n*	Case vs. control	Method	Markers	Statistical analysis
Li et al. 2020[18]	NR	OSCC patients vs. healthy controls	NR	*α-amylase, IL-6, IL-8, IL-1β, albumin, defensin-1, CD44, CD59, TNF-α, fibronectin, CRP, complement factor B, C4B, C4H, C4C, alpha-1-antitrypsin, leucine-rich alpha-2-glycoprotein 1, resistin, haptoglobin, hemopexin, serotransferrin, transthyretin, fibrinogen-β, glycoprotein α-1B, zinc finger protein 28, regulator of G protein signaling 3, indoleamine 2,3-dioxygenase, type 1 oral-facial-digital syndrome 290, kininogen 1, annexin A1, annexin A2, heat shock protein, Mac-2 binding protein, MMP-1, MMP-2, MMP-3, MMP-9, MMP-10, MMP-12, MMP-13, myeloid-related protein-14, catalase, profilin, GA module complexed with human serum albumin, endothelin 1, CRP precursor, keratin 2, keratin 10, galectin-7, phosphoglycerate mutase 1, cofilin, plakoglobin, myosin light chain 2, myosin light chain 3, fatty acid-binding protein, nucleoside-diphosphate kinase, alpha-fetoprotein, retinoic acid-binding protein 2, CA125, tissue polypeptide-specific antigen, carcinoembryonic antigen, basic fibroblast growth factor, nicotinamide N-methyltransferase, enolase-1, lactic acid*	NR
Ueda et al. 2021[52]	133	patients with OSCC or OPMD vs. healthy controls	microarray analysis and RT-PCR	*CCL20*	0.069 was used as the cut-off value for AUC
Ferrari et al. 2021[12]	NR	OSSC patients vs. healthy controls or patients with OPMD	NR	*GRO, VEGF, IL-17, IP-10, TNF-α, IL-1β, IL-1RA, IFN-γ, IL-6, IL-8, MIP-1β*	NR
Chiamulera et al. 2021[37]	1,670	patients with OPMD, OLP or periodontitis vs. healthy controls	ELISA, meta-analysis to consolidate the data	*IL-1, IL-1α, IL-1β, IL-1RA, IL-4, IL-6, IL-8, IL-10, IL-13, TNF-α*	*SMD* was used in the meta-analysis
Hussein et al. 2018[20]	567	OSCC patients vs. healthy controls	ELISA	*AMDL DR-70, SCC, SCCA-1, CA125, Cyfra 21-1, CA19-9, IL-1α, IL-6, IL-8, TPS, COL5A1, CEA, adenosine deaminase, VEGF-A, TNF-α, adiponectin, syndecan-1, ABCG1, MMP-1, prolactin, EGF, TPS, IGF-1, FN1*	A limited number of studies have employed diagnostic meta-analysis, demonstrating sensitivity and specificity levels ranging from 75% to 100%.
Manzano-Moreno et al. 2021[51]	NR	OSCC patients vs. healthy controls or patients with OPMD	NR	*miRNAs (-24, -3P, -412-3p, -512-3p, -302b-3p, -517b-3p, -134, -486-5p, -4484, -10b-5p, -200a, -365, -21, -145, -93, -184, -31, -412-3p, -34a), IL-6, IL-8, IL-1β, TNF-α, MMP-9, IL-1-RA, IL-10, 8-OHdG*	NR
Hema Shree et al. 2019[22]	308 healthy individuals and 340 patients with OSCC	OSCC patients vs. healthy controls	ELISA, Luminex bead-based multiplex assay, RT-PCR, electro-chemiluminescence immunoassay	*miRNAs (-21, -145, -184, -27B* (overexpressed), *-136* (underexpressed)), *N-leucine+N-phenylalanine, Cyfra 21-1, MMP-9, chemerin, choline+betaine+pipecolic acid+L-carnithine, IL-1β, IL-6, IL-8, TNF-α*, and others	AUC < 0.7
Diesch et al. 2021[23]	2,407 participants from 34 trials, including 995 healthy controls	OSCC patients vs. healthy controls	ELISA, Luminex bead-based xMAP®, bead-based flow cytometry	*IL-1, IL-1-RA, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-15, IL-17A, IFN-α, IFN-β, IFN-γ, TNF (-α, -β, -γ), TNF receptor 2, osteopontin, IP-10, GM-CSF, VEGF, MCP-1, sIgA*	NR

8-OHdG – 8-hydroxy-2′-deoxyguanosine; *ABCG1* – ATP binding cassette subfamily G member; AUC – area under the curve; *C4B* – complement component 4B; *CA125* – carcinoma antigen 125; *CCL20* – chemokine ligand 20; *CEA* – carcinoembryonic antigen; *CI* – confidence interval; *CRP* – C-reactive protein; *EGF* – epidermal growth factor; ELISA – enzyme-linked immunosorbent assay; *FN1* – fibronectin 1; *GM-CSF* – granulocyte-macrophage colony-stimulating factor; *GRO* – growth-regulated oncogene; *IGF-1* – insulin-like growth factor; *IL* – interleukin; *IP-10* – interferon gamma-induced protein 10; *MCP-1* – monocyte chemoattractant protein-1; *MIP* – macrophage inflammatory protein; *MMP* – matrix metalloproteinase; NR – not reported; OLP – oral lichen planus; OSCC – oral squamous cell carcinoma; OPMD – oral potentially malignant disorder; *SCCA-1* – squamous cell carcinoma antigen 1; *sIgA* – secretory immunoglobulin A; *SMD* – standardized mean difference; *TNF* – tumor necrosis factor; *TPS* – terpene synthase family; qRT-PCR – quantitative real-time reverse-transcription polymerase chain reaction; *VEGF* – vascular endothelial growth factor.

Effects on cancer	Diagnostics	Prognosis
The evaluated biomarkers exhibited either an increase or a decrease in OSCC patients when compared to healthy controls.	All markers were used to diagnose OSCC at earlier stages.	NR
NR	The positive predictive value and specificity of *CCL20* (0.983 and 0.979, respectively) showed satisfactory precision for patients with OSCC compared to those with OPMD and healthy volunteers.	The expression level of *CCL20* exhibited suboptimal sensitivity and negative predictive value.
The levels of *IL-6*, *IL-8*, *TNF-α*, and *IL-1RA* increase regularly as the condition transitions from well-differentiated to poorly-differentiated OSCC. This finding suggest a potential association between these cytokines and the aggressiveness and severity of the disease.	A comparison of the saliva samples from patients with OSCC with those from healthy controls revealed significant disparities in the concentrations of *IL-6*, *IL-8* and *TNF-α*. The concentrations of *IL-6*, *TNF-α*, *IL-8*, *IL-1β*, *MIP-1β*, *IFN-γ*, and *GRO* are higher in patients with early stage OSCC (stage I/II or T1/T2) compared to the controls. The levels of salivary *IL-6*, *IL-8* and *TNF-α* in patients with OPMD are comparatively lower than those in patients with OSCC. A statistically significant difference was observed among the cytokines when compared to the control group.	Based on the results of the surgical excision of OSCC, longitudinal studies reveal that cytokines such as *VEGF*, *MIP-1β*, *IP-10*, *IL-6*, *IL-8*, *IL-1β*, and *IL-17* are more concentrated in the presence of OSCC than at the post-surgical level.
NR	According to a meta-analysis that assessed the levels of salivary carcinogens in patients with oral carcinoma and healthy controls, a significant increase in the levels of *IL-8* (*SMD* = 1.77; 95% *CI*: 0.79–1.55), *IL-6* (*SMD* = 2.08; 95% *CI*: 1.33–2.84), *TNF-α* (*SMD* = 2.04; 95% *CI*: 0.47–3.61), *IL-1β* (*SMD* = 0.78; 95% *CI*: 0.44–1.13), and *IL-10* (*SMD* = 0.46; 95% *CI*: 0.05–0.86) were observed in the cancer group. The only salivary carcinogen without a significant difference was *IL-1α* (*SMD* = 2.21; 95% *CI*: 0.36–4.77). The study did not include *IL-1*, *IL-1RA*, *IL-4*, or *IL-13* because there was only 1 observation for each of these factors.	NR
In patients diagnosed with OSCC, there were observed alterations in the levels of *AMDL DR-70*, *SCCA-1*, *CA125*, *CA19-9*, *TPS*, *CEA*, *SCC*, *Cyfra 21-1*, *adenosine deaminase*, *adiponectin*, *MMP-1*, *IL-6*, *syndecan-1*, *prolactin*, *TPS*, *VEGF-A*, *TNF-α*, *COL5A1*, *EGF*, *IGF-1*, *IL-1α*, *IL-8*, *ABCG1*, and *FN1*. As the disease progresses, these levels undergo changes.	The OSCC groups exhibited elevated levels of *adenosine deaminase*, *adiponectin*, *IL-6*, *IL-1α*, *VEGF-A*, *TNF-α*, *COL5A1*, *ABCG1*, *MMP-1*, *IL-8*, *FN1*, *SCCA-1*, *CA125*, *CA19-9*, *TPS*, *CEA*, *SCC*, and *Cyfra 21-1* in comparison to the healthy controls.	*Prolactin* demonstrated 100% sensitivity and specificity. *Adiponectin* levels decreased as the disease progressed. Other markers (*AMDL DR-70*, *syndecan-1*, *IL-1α*, *IL-6*, *IL-8*, *TPS*, *EGF*, *IGF-1*, *VEGF-A*, and *TNF-α*) exhibited an increase in the concentration concomitant with the progression of the disease.
NR	*MicroRNAs* (*-21*, *-145*, *-93*, *-184*, *-31*, *-412-3p*, *-34a*) act as tumor activators. Higher levels of *IL-6*, *IL-8*, *IL-10*, *IL-1β*, *IL-1-RA*, *MMP-9*, *8-OHdG*, and *TNF-α* were detected in early cancer patients when compared with healthy individuals.	Correlations have been identified between *miRNAs* (*-24*, *-3p*, *-412-3p*, *-512-3p*, *-302b-3p*, *-517b-3p*, *-134*, *-486-5p*, *-4484*, *-10b-5p*, *-200a*, and *-365*) and OSCC, histological type and/or disease stage. As the condition progresses, the levels of *IL-6*, *IL-8*, *IL-1β*, *IL-1-RA*, *IL-10*, *TNF-α*, *MMP-9*, and *8-OHdG* increase.
NR	Highly sensitive salivary biomarkers for detecting OSCC include *MMP-9* and *choline+betaine+pipecolic acid+L-carnithine*. The narrow confidence level for *MMP-9* was 0.95 (0.88–1.00), whereas the narrow confidence range for *chemerin* was 1.00 (0.78–1.00). Highly precise biomarkers for OSCC included *MMP-9* (specificity: 100%), *chemerin* (specificity: 100%), overexpressed *miRNA-136* (specificity: 0.88 (0.69–0.97)), and underexpressed *miRNA-27B* (specificity: 1.0).	NR
Analyzed markers are either increased or decreased in OSCC patients when compared to healthy individuals.	NR	*TNF-α*, *IL-1β*, *IL-2*, *IL-6*, and other pro-inflammatory cytokines have been associated with the severity of oral mucosal tissue damage in cancer patients.

IL-6

Interleukin-6 is a highly sensitive and specific marker. Eighty percent of the analyzed studies have used *IL-6* as a diagnostic and prognostic biomarker. Interleukin-6 is a multifunctional cytokine that controls inflammatory reactions.[10] It aids in the development, migration, invasion, growth, proliferation, apoptosis, progression, angiogenesis, and differentiation of tumor cells. Moreover, *IL-6* stimulates the phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase (MAPK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways.[24] Through the JAK/STAT pathway, IL-6 activates STAT3 by binding to IL-6R, which then forms a complex with gp130. Furthermore, IL-6 can help tumor cells elude immune monitoring, preventing tumor rejection, given its immunosuppressive properties.[39]

IL-8

In the majority of the included studies, *IL-8* has been analyzed along with *IL-6* and regarded as the most predictive salivary biomarker. Chiamulera et al. performed a meta-analysis that compared the concentration of salivary cytokines in patients with OSCC to those of healthy controls.[37] The results demonstrated a significant increase in the level of *IL-8*, as indicated by standardized mean difference (*SMD*) of 1.77 (95% confidence interval (*CI*): 0.79–1.55).[37] The development of OSCC is influenced by the pro-inflammatory and pro-angiogenic *IL-1*, *IL-6*, *IL-8*, and *TNF-α*, which support cell survival and proliferation.[12] They activate pro-cell cycle regulators, including the nuclear factor kappa B (NF-κB), STAT proteins and MAPK/extracellular signal-regulated kinase (ERK) pathways.[40]

TNF-α

Tumor necrosis factor alpha has been utilized as a salivary biomarker in numerous studies, exhibiting high sensitivity and specificity. A meta-analysis conducted by Chiamulera et al. demonstrated a *SMD* of 2.04 (95% *CI*: 0.47–3.61) for *TNF-α* in patients with OSCC, when compared to healthy individuals.[37]

Tumor necrosis factor alpha is a multifunctional cytokine that plays a crucial role in various biological processes, including cell survival, proliferation, differentiation, and death.[41] Inflammatory cells release TNF-α, a pro-inflammatory cytokine that may contribute to inflammation-related carcinogenesis.[42] The cytokine affects various signaling pathways, including NF-κB and c-Jun N-terminal kinase (JNK). NF-κB is a key antiapoptotic cell survival signal, whereas persistent JNK activation promotes cell death. The interaction between NF-κB and JNK exerts an influence on the cellular responses to TNF-α.[37]

Tumor necrosis factor alpha has a dual role in cancer progression.[41] While it can enhance the development, proliferation, invasion, and angiogenesis of cancer cells, presumably acting as an endogenous tumor promoter,[43] it also has the potential to eradicate cancer.[41] Tumor necrosis factor alpha has been shown to induce cancer cell death, suggesting its potential in cancer treatment. However, significant research is necessary to mitigate the toxicity of TNF-α for routine administration.[44] Recent studies have focused on combination therapy, aiming to decrease survival signals such as NF-κB to enhance the susceptibility of cancer cells to TNF-α-induced apoptosis.[43]

miRNAs

In the nucleus, RNA polymerase II synthesizes long, single-stranded RNA molecules called primary microRNAs (pri-miRNAs).[45] These pri-miRNAs undergo sequential processing. Initially, they are processed by the Drosha complex in the nucleus to form precursor miRNAs (pre-miRNAs). Subsequently, the Dicer complex, located in the cytoplasm, produces mature, short, single-stranded microRNAs (miRNAs). The concentration of these mature miRNAs determines their ability to regulate the stability or translation of messenger RNA (mRNA), depending on their sequence complementarity to the target mRNA.[46]

MicroRNAs can be selectively packed in extracellular vesicles or released in bodily fluids as cell-free miRNAs linked to RNA binding proteins.[47] Additionally, miRNAs play a role in the regulation of several biological processes, including cell differentiation, proliferation, apoptosis, and the development of embryos and tissues.[48] Numerous miRNAs are involved in the regulation of bone metabolism.[49] Their expression is typically tumor-specific and is not altered by bodily fluids or tissues.[50] Furthermore, they function as the primary regulators of gene expression, making them crucial for the identification of early stages of malignant transformation.[50] As a result, the study of miRNA profiles in cancer patients offers a novel approach to the development of biomarkers for the clinical diagnosis of this illness.[51]

Additionally, the expression of certain miRNAs, such as *miR-24*, *miR-3P*, *miR-412-3p*, *miR-512-3p*, *miR-302b-3p*, *miR-517b-3p*, *miR-134*, *miR-486-5p*, *miR-4484*, *miR-10b-5p*, *miR-200a*, *miR-365*, *miR-21*, *miR-145*, *miR-93*, *miR-184*, *miR-31*, *miR-412-3p* and *miR-34a*, has been shown to be either upregulated or downregulated in OSCC patients when compared to healthy controls or patients with oral potentially malignant disorders (OPMDs).

CCL20

The expression of a small cytokine belonging to the CC chemokine family, CCL20, may play a role in the growth and dissemination of OSCC.[52] According to a study by

Ueda et al., in which 0.069 was used as a cut-off value for the area under the curve (AUC), the researchers found that the specificity and positive predictive value of *CCL20* were 0.983 and 0.979, respectively, demonstrating satisfactory accuracy for patients with OSCC as compared to those with OPMD and healthy volunteers.[52] According to the microarray analysis, *CCL20* mRNA was found to be strongly accumulated in the saliva of OSCC patients. Using quantitative real-time reverse-transcription polymerase chain reaction (qRT-PCR), it was ascertained that *CCL20* expression was considerably higher in the saliva of patients with OSCC compared to healthy volunteers and patients with OPMD ($p < 0.001$).[52]

MMPs

Matrix metalloproteinases are a group of structurally similar zinc-containing endopeptidases.[53] A total of 23 MMPs have been documented. Numerous cell types, including immune cells, epithelial cells and fibroblasts, generate MMPs.[37] The extracellular matrix (ECM) components that are degraded by MMPs result in altered cell–matrix and cell–cell interactions as well as the activation or inactivation of cytokines, growth factors and cell surface receptors.[54]

Collagenase-1, also known as MMP-1, is the earliest-identified member of the MMP family and plays a pivotal role in ECM remodeling. It is strongly associated with tumor metastasis, angiogenesis, and inflammation.[55] MMP-1 is synthesized as an inactive zymogen (pro-MMP-1) and secreted via a signal peptide-guided pathway. Its activation is triggered by the proteolytic removal of the pro-peptide domain, typically by a serine protease or another MMP, such as MMP-3.[56] This activation exposes the catalytic site, enabling MMP-1 to degrade several ECM components, including fibronectin, gelatin and laminin, thereby contributing to ECM remodeling.[57]

The activity of MMP-1 is controlled by the binding of tissue inhibitors of metalloproteinases (TIMPs) or by autolytic cleavage.[53,58] The equilibrium between the concentration of active metalloproteinases and their inhibitors (TIMPs) may become imbalanced and result in pathological alterations linked to uncontrolled ECM turnover, tissue remodeling, inflammatory response, cell proliferation, and migration.[54,56]

Collagen type IV, the predominant glycoprotein component of the basement membrane and a factor in the control of inflammatory and vascular processes, is destroyed by MMP-2 (collagenase-4).[58] Numerous elements of ECM, including proteoglycans, fibronectin, laminin, and collagen types III, IV and V, are broken down by stromelysin, also known as MMP-3.[59] Type II collagen is best broken down by MMP-13 (collagenase-3).[58]

Type IV collagen, a significant component of the basal lamina, as well as other types of collagen (V, VII and X), elastin and fibronectin are all degraded by the MMP-9 class of zinc-dependent proteinases,[12] which have been linked to a variety of clinical diseases. According to studies,[58,59] stromal cells surrounding the invading front of metastatic tumors exhibit elevated expression of MMP-9. Matrix metalloproteinase-9, along with MMP-2, belongs to the gelatinase subgroup of the MMP family. The overexpression of MMP-9 has been frequently observed in a variety of malignant tumors. Eighty percent of studies included in this review have analyzed *MMP-9* as a biomarker, emphasizing its sensitivity and specificity.

Conclusions

This systematic review highlights the growing potential of salivary biomarkers in the early detection and prognostic evaluation of OSCC. The analysis identifies several promising candidates that may serve as non-invasive, reliable tools for clinical application. However, to facilitate their integration into routine clinical practice, future research should focus on large-scale, longitudinal, and multicenter studies aimed at validating these biomarkers and establishing a robust, standardized salivary biomarker panel for OSCC diagnosis and prognosis.

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.

ORCID iDs

Gayathri Rengasamy https://orcid.org/0000-0003-3325-7593
Hema Shree Kasirajan https://orcid.org/0009-0000-7447-2623
Vishnu Priya Veeraraghavan https://orcid.org/0000-0002-5071-9860
Pratibha Ramani https://orcid.org/0000-0002-5426-3506
Gabriele Cervino https://orcid.org/0000-0003-4619-4691
Giuseppe Minervini https://orcid.org/0000-0002-8309-1272

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Review

Efficacy of mechanical tongue cleaning on taste perception: A systematic review

Fulvia Costantinides[A,C,E,F], Marta Gionechetti[B,D,F], Monica Baiana[C,F], Erica Vettori[C,E,F], Vanessa Nicolin[C,F], Roberto Di Lenarda[E,F]

Department of Medicine, Surgery and Health Sciences, University of Trieste, Italy

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):361–369

Address for correspondence
Fulvia Costantinides
E-mail: f.costantinides@fmc.units.it

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on August 31, 2023
Reviewed on September 13, 2023
Accepted on September 25, 2023

Published online on April 17, 2025

Abstract

The aim of the present literary review was to compile the most recent evidence regarding the impact of mechanical tongue cleaning on gustatory perception in patients with a coated tongue. The present study adhered to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The bibliographic survey of PubMed®, Scopus, Web of Science, and Cochrane Library databases was conducted using the following combination of keywords and Boolean operators: (tongue brushing AND taste) OR (tongue cleaning AND taste) OR (tongue coating AND taste). The quality of studies and the risk of bias were assessed based on the checklist provided by Downs and Black.

Four articles were selected for the review based on the established inclusion criteria. The analysis of the data showed a decrease in lingual coating post-brushing in all studies. All articles demonstrated an improvement in gustatory sensitivity following mechanical removal of the lingual patina, though not every study observed a statistically significant increase for the same flavor. The outcomes of this review suggest that mechanical cleaning of the dorsum of the tongue can increase gustatory perception, therefore, it could be considered a promising and cost-effective addition to daily oral hygiene practices.

Keywords: taste, brushing, lingual coating, tongue cleaning

Cite as
Costantinides F, Gionechetti M, Baiana M, Vettori E, Nicolin V, Di Lenarda R. Efficacy of mechanical tongue cleaning on taste perception: A systematic review. *Dent Med Probl.* 2025;62(2):361–369. doi:10.17219/dmp/172888

DOI
10.17219/dmp/172888

Highlights

- Lingual coating has an influence on gustatory perception.
- Literature reports improved taste sensitivity following mechanical removal of lingual coating.
- Enhanced perception of salty taste may contribute to reduced dietary intake of sodium chloride.
- Lingual cleansing is associated with systemic health benefits, cardiovascular disease prevention, and is an effective, low-cost addition to daily oral hygiene practices.

Introduction

Taste is considered one of life's most profound pleasures, and the ability to savor a food or drink can provide a sense of fulfillment and general satisfaction in humans, thereby fostering a positive mood. Gustatory sensitivity plays an important role in the quality of life for all living beings. In fact, an altered taste sensation can have a negative impact on a person's oral and systemic health. Such alterations can lead to changes in appetite, potentially resulting in unhealthy food choices and compromising the individual's nutritional status. For example, elevated salty or sweet taste thresholds can lead to the overconsumption of foods rich in sodium or sugars, which can further contribute to the development and/or progression of cardiac pathologies, hypertension, obesity, and diabetes mellitus.[1]

The gustatory pathway is initiated by the interaction of non-volatile, saliva-soluble chemicals with the taste receptors located on the villi of the gustatory pore. Once the quantity of the solution reaches a certain concentration threshold (protopathic threshold), a stimulus is created that triggers the transduction mechanism of the electrical signal. The transduction process is multifaceted, depending on the chemical nature of the substance. For instance, acids and salts are associated with receptors coupled with membrane ion channels, while sweet, bitter and umami substances are correlated with G protein-coupled receptors.[2–4]

Numerous tests are currently available to evaluate gustatory perception and gustatory disorders. These exams mainly consist of chemical, electrical[5–11] and imaging techniques.[12] Chemical evaluations are used for the assessment of taste, while electrical and imaging tests are used to diagnose taste disorders.

The alteration of gustatory perception can be influenced by various systemic pathologies, as well as by drug intake, chemotherapy and radiotherapy treatments, zinc deficiency, smoking habits, and advancing age. Despite the recognition of this issue, there is a paucity of effective therapeutic strategies to address it.[13]

In addition to the scarce therapies available, it must be acknowledged that the dorsum of the tongue is usually covered by the coating, a physiological whitish patina, which is interposed between the taste buds and the food molecules introduced into the oral cavity.[14]

In order to elicit the effect of gustatory perception, the non-volatile molecules introduced into the oral cavity must penetrate the taste buds present on the lingual papillae and bind to the respective taste receptors. This step could be hindered by the presence of a coating. The question that naturally arises is whether a simple mechanical maneuver of cleansing the lingual back, which involves the reduction of the physiological coating, can have effects on the perception of taste, improving it. Several studies have validated the effectiveness of mechanical tongue cleaning in reducing the coating.[15–20] A systematic review has demonstrated that cleaning the tongue with a scraper or tongue cleaner is effective in decreasing the lingual coating.[21] Additionally, a study conducted on a sample of 20 subjects who tested a plastic scraper, a metal scraper and a tongue cleaner brush, revealed a substantial decrease in the coating detected by the Winkel tongue coating index (WTCI). In this case, the optimal outcome was achieved with the plastic scraper, resulting in an approx. 55% reduction of the coating.[22]

The aim of this systematic review was to evaluate the impact of mechanical tongue cleaning on taste perception in patients with lingual coating.

Material and methods

Search strategy

A literature review was conducted to identify the most valid evidence regarding the impact of mechanical tongue cleaning on gustatory perception in patients with tongue coating. The study design adhered to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) protocol as updated in 2020.[23] Previous systematic reviews were not used as a model as they are not available in the literature.

The bibliographic search was conducted in May and June 2021, retrospectively covering a 20-year period from 2001 to 2021. The search was carried out in the PubMed®, Scopus, Web of Science, and Cochrane Library databases.

The electronic version of the documents was obtained using the EZproxy service provided by the University Library System of the University of Trieste, Italy. Two researchers (MG and FC) independently selected the titles and abstracts of the studies identified in the electronic databases.

Eligibility criteria and study selection

The following research question was formulated: "Can the removal of a lingual coating by mechanical cleaning affect taste perception?".

To formulate the research question in a structured way, the PICO method was taken into consideration. This method relies on 4 elements: the P factor (problem/patient/population); the I factor (intervention); the C factor (comparison/control); and the O factor (outcomes).[24]

The model developed for this study did not take the element C into consideration, i.e., comparison/control. In fact, the main objective of this study was not to evaluate which tool or mechanical technique is most effective in promoting gustatory perception and not even to compare mechanical cleansing with other strategies such as chemical ones, but rather to verify if there is an interaction between physical removal of the coating and gustatory perception.

To conduct this literature review, the PIO model was constructed (Table 1). The keywords mentioned in Table 1 have been combined to form the following search algorithm: (tongue brushing AND taste) OR (tongue cleaning AND taste) OR (tongue coating AND taste).

Study inclusion and exclusion criteria

The inclusion criteria for this review encompassed meta-analyses, systematic reviews, randomized and non-randomized controlled trials, uncontrolled clinical trials, and prospective cohort studies written in English and published from 2001 to 2021, with no time limit on their duration. The participants of the studies included individuals of both sexes, older age, smokers and non-smokers, with the presence of a coating on the lingual back. The intervention described in the studies involved the detection of the lingual coating pre- and post-mechanical cleaning as well as cleansing of the lingual back with any mechanical technique and with any tool suitable for such use (a tongue cleaning brush, a tongue scraper, an electric toothbrush with a special insert, or a manual toothbrush), which resulted in the detection of at least 1 taste using taste tests (filter paper disc method, taste tablets, taste strips, or drop method).

The analysis excluded single case studies, case series, pilot studies, preliminary studies, retrospective cohort studies, expert comments and opinions, editorials, studies that were not completed because they had been still under development, as well as articles not viewable as full texts and those with a sample of less than 10 subjects. Additionally, studies that evaluated patients with cognitive deficits or those who did not recognize at least 1 of the tested tastes were excluded to ensure the elimination of subjects with pathologies that involve an evident loss of taste or who take drugs that predominantly affect the perception of taste.

Qualitative evaluation of studies and the risk of bias

To evaluate the methodological quality of the studies and to provide an overall score for the validity of each individual research, a checklist was developed following some of the indications provided by Downs and Black (Table 2).[25] Each article was subjected to the checklist individually, and based on the achieved score, the quality of the studies was classified as low (≤16), average (17–18) or high (19–20).

Results

The search yielded 264 articles, as follows: 84 articles were identified in PubMed®; 34 papers in Scopus; 38 studies in Cochrane Library; and 108 articles in Web of Science. Studies appearing in more than 1 database were considered only once.

All duplicate articles were eliminated, resulting in a total of 182 papers. Following a thorough examination of titles and abstracts, 172 publications were excluded as they were not pertinent to the research objective. Any disagreements between researchers were resolved through discussion. The 10 remaining articles were then subjected to an analysis according to the established inclusion and exclusion criteria.

One article was excluded due to its language, which was German. One pilot study and one preliminary study were excluded due to the low level of scientific evidence and because they did not meet the inclusion criteria of the review. Two publications were not considered because they did not report the measurements of gustatory perception before and after mechanical cleaning of the dorsum of the tongue. Finally, 1 article was excluded from the review because its text was not viewable.

At the end of the search process, 4 articles satisfied the inclusion and exclusion criteria established for this systematic review (Fig. 1, Table 3).[13,26–28]

Table 1. PIO model constructed for the study (adapted from PICO)

Factor	Description	Keywords
P (problem/patient/population)	patients with lingual coating	tongue coating
I (intervention)	mechanical cleaning of the dorsum of the tongue	tongue brushing, tongue cleaning
O (outcomes)	effects on taste perception	taste

Table 2. Checklist for the assessment of the quality and risk of bias of the studies included in the review, based on the study by Downs and Black[25]

No.	Item	Score [points]
1	study design	RCT: 3 CCT: 2 UCCT, PCS: 1
2	objective/aim of the study clearly described	yes: 1 no: 0
3	clear description of the results in the abstract	yes: 1 no: 0
4	description of the patients included in the study based on 6 criteria: sample size; age; sex; general health; smoking status; presence/absence of the lingual coating	partial description: 1 complete description: 2
5	evidence for the type of index used for coating detection	yes: 1 no: 0
6	description of the mechanical cleaning of the dorsum of the tongue based on 2 criteria: type of device; technique adopted	partial description: 1 complete description: 2
7	description of taste detection based on 3 criteria: type of test adopted; type of taste analyzed; chemical concentration of the solution used for taste detection	partial description: 1 complete description: 2
8	results organized in graphs and/or tables showing the output variables before and after mechanical tongue cleaning	yes: 1 no: 0
9	sample recruited in the same time period	yes: 1 no: 0
10	same duration of the follow-up in the whole sample	yes: 1 no: 0
11	no drop-outs at follow-up	yes: 1 no: 0
12	patients blinded to the type and concentration of taste administered	yes: 1 no: 0
13	following the established protocol in each phase of the study	yes: 1 no: 0
14	type of statistical analysis	inferential: 2 descriptive: 1
15	reporting *p*-values (when <0.05 or <0.001) for statistically significant results	yes: 1 no: 0

RCT – randomized controlled trial; CCT – controlled clinical trial; UCCT – uncontrolled clinical trial; PCS – prospective cohort study.

Results of individual studies

Participants

Two studies considered a sample of 90 subjects,[26,27] 1 study included 65 individuals,[13] and another encompassed 16 subjects.[28] All studies included both male and female participants, except for the study conducted by Quirynen et al., in which the sex of the participants was not specified.[28] The age of the participants varied from 21 to 94 years. In particular, the study by Ohno et al. focused on a sample of elderly subjects, aged 65–94 years.[27] Quirynen et al. evaluated subjects with an age range between 21 and 50 years, while the other 2 studies included a wider age range.[13,26,28] Seerangaiyan et al. and Quirynen et al. examined only non-smokers, whereas studies by Timmesfeld et al. and Ohno et al. did not specify this feature.[13,26–28]

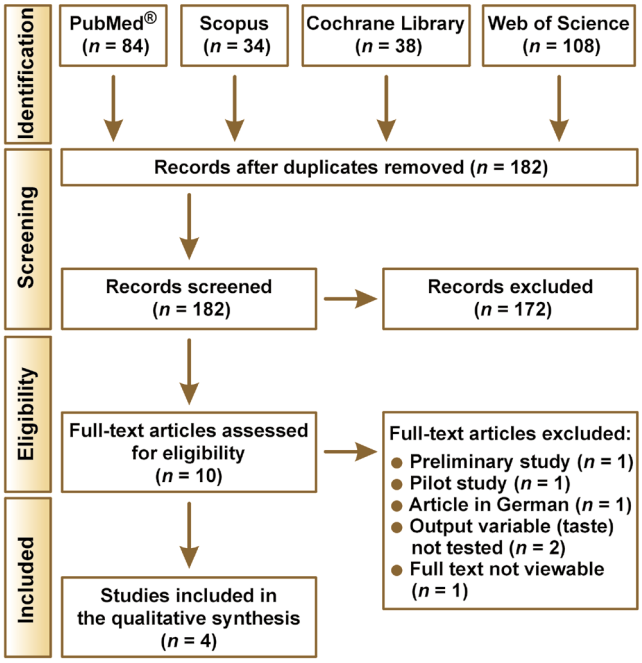

Fig. 1. Flowchart of the search strategy

Table 3. Studies included in the systematic review

Study	Title	Study design
Timmesfeld et al. 2021[13]	Mechanical tongue cleaning is a worthwhile procedure to improve the taste sensation	UCCT
Seerangaiyan et al. 2018[26]	Tongue cleaning increases the perceived intensity of salty taste	UCCT
Quirynen et al. 2004[28]	Impact of tongue cleansers on microbial load and taste	RCT
Ohno et al. 2003[27]	Improvement of taste sensitivity of the nursed elderly by oral care	RCT

Technique and duration of tongue cleaning

The participants of the studies by Timmesfeld et al. and Seerangaiyan et al. used a tongue scraper.[13,26] Two other studies incorporated a control group: one compared the scraper with a toothbrush[28]; the other compared the scraper with a water rinse.[27] Quirynen et al. and Timmesfeld et al. instructed participants to perform tongue cleansing maneuvers 2 times a day for 14 days. In contrast, the studies by Seerangaiyan et al. and Ohno et al. involved a single cleansing session, with the coating and taste indices being detected 20 min later.[13,26–28]

Table 4. Summary of the studies included in the review

Study	Sample size and sex distribution	Inclusion and exclusion criteria	Age [years]	Brushing technique	Coating assessment technique	Taste recording technique	Amount of coating remaining after tongue cleaning	Taste perception after tongue cleaning
Timmesfeld et al. 2021[13]	65 patients: 27 males, 38 females (50 smokers and 15 non-smokers)	IC: healthy subjects EC: patients with lost or reduced taste perception	21–91	tongue scraper twice a day for 14 days	WTCI: 0 = no coating 1 = moderate coating 2 = massive coating (score range: 0–12) subdivision of the tongue in 6 areas	16 taste strips: 4 tastes with 4 concentrations score: 0–4, maximum score: 16	• reduction in coating among non-smokers ($p < 0.001$) and smokers ($p = 0.016$) • better results for smokers compared to non-smokers • no differences in terms of age and sex	• significant improvement in the total taste ($p < 0.001$) and sour taste perception ($p < 0.001$) among non-smokers; total taste improved, especially in older patients ($p = 0.01$) • significant improvement in the total taste ($p = 0.031$) and sour taste perception ($p = 0.41$) among smokers; no differences in terms of age
Seerangaiyan et al. 2018[26]	90 patients: 29 males, 61 females	EC: chronic pathologies, smoking, intake of drugs with an effect on taste perception, pregnancy, food allergies	25–70	tongue scraper	WTCI: 0 = no coating 1 = moderate coating 2 = massive coating (score range: 0–12) subdivision of the tongue in 6 areas, final coating test performed 20 min after brushing	filter paper disc method: salty taste, 1 concentration (1.6 g of NaCl (27%)) VAS: 0–10, 1 = not salty, 10 = very salty	• reduction in coating • no differences in terms of age and sex	• significant improvement in the perception of salty taste ($p = 0.0002$) in 65% of males and 59% of females • reduction in the perception of salty taste in 14% of males and 18% of females • lack of variation in salty taste perception in 21% of males and 23% of females
Quirynen et al. 2004[28]	16 patients randomly divided into 2 groups, sex distribution unspecified	IC: healthy periodontal tissues EC: smoking, intake of antibiotics in the preceding 6 months	21–50	2 groups: • toothbrush twice a day for 14 days • tongue scraper twice a day for 14 days	MTCI: 0 = no coating 1 = coating <⅓ of the dorsum of the tongue 2 = coating <⅔ of the dorsum of the tongue 3 = coating >⅔ of the dorsum of the tongue (score range: 0–12) subdivision of the tongue in 4 areas	drop method: 4 tastes with 3 concentrations	• reduction in coating ($p < 0.001$) • higher reduction in the anterior area of the tongue • no differences between the cleaning methods (scraper vs. toothbrush)	• slight improvement in the total taste perception after tongue cleaning with both the scraper and the toothbrush • significant improvement in the perception of salty taste ($p = 0.008$) and bitter taste ($p < 0.003$) after tongue cleaning with the scraper; borderline result for sweet taste ($p = 0.06$)
Ohno et al. 2003[27]	90 patients: 28 males and 62 females, divided into 2 groups of 50 subjects (brushing group) and 40 subjects (oral rinse control group)	EC: cognitive disorders, BMS, alterations in taste perception, mucosal pathologies of the oral cavity	65–94	2 groups: • tongue scraping by a clinical operator • control group (water rinse)	MTCI: only patients with MTCI 1 (coating <⅓ of the dorsum of the tongue) have been included subdivision of the tongue in 4 areas	drop method: 4 tastes with 13 concentrations applied with a syringe 1 = minimal concentration, 13 = maximal concentration	reduction in coating in the study group	• significant improvement in the perception of salty taste ($p < 0.05$) and sour taste ($p < 0.05$) after tongue cleaning with the scraper in older patients • no significant improvements for sweet and bitter tastes

IC – inclusion criteria; EC – exclusion criteria; BMS – burning mouth syndrome; WTCI – Winkel tongue coating index; MTCI – Miyazaki tongue coating index; VAS – visual analogue scale; NaCl – sodium chloride.

Measurement of the coating level

Two articles applied the WTCI by dividing the lingual back into 6 areas.[13,26] The other 2 studies used the Miyazaki tongue coating index (MTCI) to assign a score for the whole tongue,[27] or a partial score obtained from a division of the lingual back into 4 areas.[28] The initial coating level was measured by detection indices in all studies. The post-brushing reduction was observed by all authors, but its classification as a detection rate was only documented in the articles by Timmesfeld et al. and Quirynen et al.[13,28]

Taste test

The drop method was adopted in the studies by Quirynen et al. and Ohno et al.[27,28] In both cases, 4 tastes were tested (sweet, bitter, salty, and sour). However, in the first study, each taste solution was presented at 3 different concentrations, while in the second, 13 concentrations were used. Timmesfeld et al. employed 16 taste strips, each with 4 distinct concentrations.[13] Seerangaiyan et al. applied the filter paper disc method with a single concentration, but only for salty taste.[26] The characteristics of the included studies are summarized in Table 4.

Assessment of the risk of bias

The results of the risk of bias assessment are presented in Table 5. In all articles, the objective of the research was clearly stated, as was the description of the results obtained, which are also presented exhaustively in the abstract.

Only 1 study satisfactorily described the sample, meeting all 6 requirements outlined in the checklist.[26] The remaining articles did not specify the presence of lingual coating in the sample, coating which, however, was then detected using the WTCI and MTCI indices in all the studies presented.[26] Furthermore, in the study by Quirynen et al., the sex of the selected patients was not documented.[28]

The technique of mechanical removal of the lingual coating and the tools used have been adequately described in all articles, except for the publication by Seerangaiyan et al., where the removal methodology was not specified.[26] All the studies duly illustrated the taste detection test used and the type of taste(s) tested, as well as the degree of concentration of the taste solutions applied.

All the parameters measured before and after treatment were clearly represented by graphs and/or tables, except for the study by Seerangaiyan et al. In this study, a histogram was used to report the number of subjects whose gustatory perception increased or decreased.[26] However, the variation in this perception was not reported with respect to baseline.[26]

The participants in each individual study were recruited during the same period. The re-evaluation time following the cleansing of the lingual back was consistent for each subject in the sample. In particular, the tests were repeated 20 min after brushing[26,27] or after 2 weeks.[13,28] Patient loss was not recorded during the entire duration of the research. All studies were conducted in single-blind fashion and adhered to previously established protocols.

From a statistical perspective, the articles are of the inferential type, and thus the samples are considered representative of the entire population. In all studies, probability values with a p-value <0.05 were considered statistically significant, and actual measurements were reported, except when the p-value was <0.001. However, in the study conducted by Ohno et al., the actual probability values were not documented.[27]

The total scores obtained for each article were then compared with the quality ranges of the studies. The results indicated that 2 publications demonstrated a high quality level,[27,28] while the remaining 2 exhibited a medium level of quality.[13,26]

Discussion

The aim of the study was to determine whether the coating, not removed from the dorsum of the tongue, could interfere at the level of the gustatory pathway, thereby

Table 5. Assessment of the quality of the studies included in the review

Study	Items for quality assessment															Total	Quality
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Timmesfeld et al. 2021[13]	1	1	1	1	1	2	2	1	1	1	1	1	1	2	1	18	medium
Seerangaiyan et al. 2018[26]	1	1	1	2	1	1	2	0	1	1	1	1	1	2	1	17	medium
Quirynen et al. 2004[28]	3	1	1	1	1	2	2	1	1	1	1	1	1	2	1	20	high
Ohno et al. 2003[27]	3	1	1	1	1	2	2	1	1	1	1	1	1	2	0	19	high

preventing the interaction between the taste receptors and non-volatile substances introduced into the oral cavity. This lingual patina may negatively affect gustatory sensitivity, but this reduction in taste could also depend on other factors such as certain oral and systemic pathologies, the intake of certain types of medications, and smoking habits. To mitigate the impact of these potential confounders, the subjects included in this review were required to recognize at least one of the tested tastes. This approach was adopted to exclude individuals with evident gustatory impairment or those who were taking medications that predominantly affect gustatory perception. Adult patients, smokers and non-smokers, even those with reduced but not absent gustatory capacity, were included in this review, as its objective was to investigate whether mechanical cleansing alone could enhance gustatory sensitivity.

All of the analyzed studies adhered to a similar set of procedural steps. All subjects initially underwent a lingual coating detection test using the WTCI[13,26] or MTCI.[27,28] Subsequently, the patients were administered a chemical test to detect gustatory perception. Specifically, 2 studies adopted the drop method,[27,28] one study used the taste strips,[13] and one study employed the filter paper disc method.[26] After the preliminary assessments, the subjects were instructed to perform mechanical tongue cleansing maneuvers. At the end of the procedure, the level of lingual coating was evaluated, and the taste test was repeated. Seerangaiyan et al. and Ohno et al. performed the re-assessment approx. 20 min after brushing, while Timmesfeld et al. and Quirynen et al. made the re-assessments after 14 days.[13,26–28] The analysis of the final data shows that the post-brushing lingual coating decreased in all studies ($p < 0.05$).[13,26–28]

Regarding taste assessment tests, discordant results were found for the sweet taste. As demonstrated in the study by Quirynen et al., a borderline increase was observed ($p = 0.06$). Timmesfeld et al. reported a statistically significant increase only in smokers ($p = 0.031$).[13,28] In contrast, Ohno et al. found that the sweet taste did not exhibit a considerable positive variation ($p > 0.05$).[27]

The perception of salty taste was significantly improved in studies by Quirynen et al. ($p = 0.008$), Ohno et al. ($p < 0.05$), and Seerangaiyan et al. ($p = 0.0002$).[26–28] The latter study tested only sodium chloride on a sample of 90 subjects (non-smokers), 29 males and 61 females. The authors found that 65% of males and 59% of females reported an increase in the perception of salty taste, while 14% of males and 18% of females noted a reduction. The remaining participants exhibited no change after the removal of the lingual coating. Seerangaiyan et al. ascribed the negative differences observed to 2 factors: the brief interval (20 min) between the physical stimulus exerted with the scraper and the taste test; and the varying levels of force applied by each subject during the mechanical tongue cleaning.[26] In the first case, the lingual scraping could have caused a momentary stimulation that would have interfered with gustatory perception. In the second case, excessive pressure could have stimulated the trigeminal nerve, negatively impacting the gustatory pathway. Ohno et al. concurred that aggressive brushing could cause adverse effects on taste sensitivity, resulting in damage to the lingual papillae.[27]

The variation in the perception of the salty taste was the most frequently tested, and 1 study focused exclusively on this taste. It was positively evaluated in 3 out of 4 studies. The observed increase in the perception of sodium chloride after brushing has the potential to positively influence patients' eating habits, prompting them to consume less salty foods. Excessive salt intake – beyond the daily threshold of 5 g as recommended by the World Health Organization (WHO) – represents a risk factor for the development and/or exacerbation of various pathologies, including cardiovascular disease, hypertension, stroke, obesity, kidney disease, and gastric cancer. Therefore, the improvement in the perception of salty taste after mechanical cleansing assumes significant importance, especially given that the average daily consumption of salt typically doubles the amount recommended by the WHO guidelines.[26]

Another aspect highlighted by Ohno et al. concerns the physiological decline of common gustatory sensitivity with age.[27] The authors state that the increase in gustatory perception observed in their study, after mechanical cleansing, is particularly significant for elderly individuals. This enhancement, they posit, could facilitate a healthier lifestyle for this demographic, potentially improving their appetite and chewing. It has also been hypothesized that brushing can stimulate the dorsum of the tongue, enhancing blood flow and thus amplifying the salivary secretion at the level of the taste buds, contributing to an improvement in gustatory perception. However, according to Ohno et al., the latter aspect has not yet been fully examined and requires further investigation.[27]

Limitations

A systematic review of the literature reveals certain limitations, which were in part mentioned in the discussion. One such limitation pertains to the limited period (20 min) between brushing and the taste test. This duration, adopted in the studies by Seerangaiyan et al. and Ohno et al., may be too short to eliminate the effects of mechanical cleaning stimuli on the dorsum of the tongue.[26,27] Moreover, it could have had a negative influence on the level of gustatory perception by altering its sensitivity. Another factor that could have influenced the results is the fact that each participant in the studies examined (with the exception of the study by Ohno et al.) may have applied different levels of force during the tongue cleaning phase, thereby stimulating the trigeminal nerve in different ways.

Conclusions

This systematic review summarizes the available evidence regarding the impact of mechanical cleaning of the dorsum of the tongue on gustatory perception in patients with a coated tongue. Although there are few articles addressing this subject, all those examined confirmed an increase in gustatory sensitivity due to the mechanical removal of the lingual patina. However, not every study found a statistically significant increase for the same taste. Three out of 4 studies demonstrated an increase in salty taste sensitivity after the removal of the tongue coating.[26–28] This result could help reduce the intake of sodium chloride by limiting the risk of cardiovascular diseases.

Considering the beneficial effects of brushing on gustatory perception, also in terms of systemic health, it would be interesting to study the topic by focusing on the sweet taste; a possible increase, in fact, could contribute to a reduction in the risk of developing systemic diseases, such as diabetes and obesity. Future studies should also analyze the data based on age and smoking status of the participants, considering a follow-up of at least 2 weeks in order to allow for the cellular turnover of the taste buds. It would also be useful to compare which tool, the scraper or the toothbrush, is more advantageous in terms of gustatory perception.

In conclusion, mechanical cleaning of the dorsum of the tongue can increase gustatory perception. Therefore, it could be considered a promising and cost-effective addition to daily oral hygiene practices.

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.

ORCID iDs

Fulvia Costantinides https://orcid.org/0000-0001-6044-7531
Marta Gionechetti https://orcid.org/0009-0002-5724-8632
Monica Baiana https://orcid.org/0009-0006-5835-4428
Erica Vettori https://orcid.org/0000-0002-8478-5735
Vanessa Nicolin https://orcid.org/0000-0002-5665-6493
Roberto Di Lenarda https://orcid.org/0000-0002-9051-4413

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Review

Revisiting and rethinking on staging (severity and complexity) periodontitis from the new classification system: A critical review with suggestions for adjustments and a proposal of a new flowchart

Gustavo Vicentis de Oliveira Fernandes[1,A–F], Juliana Campos Hasse Fernandes[2,A–F]

[1] Missouri School of Dentistry and Oral Health, A.T. Still University, St. Louis, USA
[2] Private researcher, Ann Arbor, USA

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):371–391

Address for correspondence
Gustavo Vicentis de Oliveira Fernandes
E-mail: gustfernandes@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on June 25, 2024
Reviewed on August 3, 2024
Accepted on August 7, 2024

Published online on April 30, 2025

Abstract

This critical review revisited the new classification system for periodontitis, specifically for staging, suggesting modifications and introducing a new flowchart for a better clinical evaluation. It evaluated articles published between 2018 and 2024 in the English language, which had an educational motivation focused on staging periodontitis. The PubMed/MEDLINE, Web of Science and Embase databases were used to retrieve the articles. The focus questions involved the analysis of all parameters for staging periodontitis.

A total of 836 articles were initially found, of which 388 duplicates were excluded, 448 were evaluated by title and abstract, 26 articles were followed for full-text reading, and 6 articles were finally included in this critical review ($k = 0.98$). All articles included detailed parameters and steps referring to diagnosing periodontitis. Therefore, it was possible to observe instability and 'gray zones' in the staging step, which was due to the lack of priority and an organized order sequence.

This review suggests the severity parameters cannot be overcome by the complexity parameters, following a cumulative sequence: clinical attachment loss (CAL) (1st); radiographic bone loss (RBL) (2nd); tooth loss due to periodontitis (TLP) (3rd); and then the complexity parameters. An exception must be permitted only for the complexity factors between Stages III and IV that can change the initial Stage (III or IV) obtained through the severity analysis, but only between the 2 stages. Moreover, for patients without tooth loss or with TLP ≤ 4 (without the need for complex rehabilitation), and presenting any type of drifting or flaring or a secondary traumatic occlusion, there is no justification for moving the diagnosis from Stage III to Stage IV.

Keywords: periodontitis, diagnosis, classification, review

Cite as
Fernandes GVdO, Fernandes JCH. Revisiting and rethinking on staging (severity and complexity) periodontitis from the new classification system: A critical review with suggestions for adjustments and a proposal of a new flowchart. *Dent Med Probl.* 2025;62(2):371–391. doi:10.17219/dmp/192121

DOI
10.17219/dmp/192121

Introduction

Periodontitis is a plaque-induced multifactorial disease (dysbiosis) with a chronic inflammatory nature, characterized by microbially associated and host-mediated (determined by genetic, epigenetic, lifestyle, environmental, and behavioral risk factors), which is characterized by progressive destruction of structures that support the tooth, such as local bone and periodontal ligament; ultimately, at a more severe level, can cause tooth loss.[1,2] It may compromise and affect mastication, esthetics, self-confidence, and quality of life.[3] Periodontitis was listed as the 11th most prevalent condition in the world.[4] Moreover, it is known that periodontitis shares risk factors with other chronic diseases[5–7] and has bidirectional associations with general health.[8] This fact leads the clinical and scientific community to the consensus that improvements in the periodontal condition may offer benefits for systemic health and well-being.[2] Similar to many other chronic diseases, periodontitis has no cure. Then, it is paramount to do supportive periodontal therapy (SPT) ("periodontal maintenance") to prevent the progression because it is not possible to eliminate the disease and future complications.[9] For this reason, patient-risk assessment must be performed at multiple levels (patient/systemic level, mouth level, tooth, and site level).[10] The concept of risk assessment was implemented in the new Classification system for periodontal diseases.[11]

This new Classification of Periodontal and Peri-implant Diseases and Conditions, published in 2018, is one of the most complete classifications for periodontal and peri-implant diseases.[12] It was developed from the efforts of the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP) at the 2017 World Workshop. This new classification system, worldly disseminated, created a periodontitis group divided into (1) periodontitis, (2) necrotizing periodontitis, and (3) periodontitis as a result of the systemic condition. Then, Periodontitis includes staging and grading dimensions, requiring attention for many clinical parameters and radiographic examinations.[13]

The staging and grading system brings multiple levels of evaluation to help with the classification of periodontitis and to distinguish approaches to manage clinical cases better.[11] Staging aims to evaluate the severity based on the interdental clinical attachment loss (CAL) at the site of greatest loss, radiographic bone loss (RBL), tooth loss due to periodontitis; the complexity of treatment, which observes probing depth (PD), bone loss pattern (horizontal/vertical), furcation involvement, ridge defects, and the need for complex rehabilitation due to masticatory dysfunction, secondary occlusal trauma, bite collapse, drifting, or flaring; and extent and distribution of periodontitis, localized (< 30% teeth), generalized (≥ 30% teeth), or molar-incisor distribution. Grading has added another dimension and aims to determine the rate of disease progression and the response to standard periodontal therapy through RBL or CAL over 5 years, the percentage of bone loss/age, and the presence of specific risk factors (diabetes and/or smoking).[13]

The dentistry community is still undergoing the process of adaptation to this new system. Some "gray zone" cases have appeared for treatment, which may produce uncertain clinical scenarios.[14] Thereby, students, clinicians, specialists, researchers, and educators have had general difficulties adopting, understanding, teaching, and applying this new classification in the routine. The complaints turn around the difficulties in determining the stage and grade of periodontitis due to the existence of many clinical and radiographic parameters.[15] To overcome these problems, some strategic flowcharts have been published. They were considered a simple way to make decisions. They were proposed not only to facilitate the performance of fast and accurate periodontitis staging and grading but also to minimize confusion and inconsistent diagnoses.[15–17] However, they raised questions and concerns regarding some points, e.g., considering "tooth loss" as the primary criterion for the severity of periodontitis.

Therefore, many questions commonly appear similar to the implementation of any new system. However, professionals must continue applying this new classification in their routines in order to become more familiar with it. Even with this, the correct assessment of the stage and grade for periodontitis has raised a high level of concern since it is not practical for many clinicians to find and make rapid diagnoses in daily practice.[13,18,19] Then, the goal of this critical review was to revisit the new classification, specifically regarding the staging of Periodontitis, in order to clarify and discuss specific points, suggest some modifications, and introduce a new flowchart for a better clinical evaluation of the periodontitis.

Methods

This critical review evaluated the articles published after the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions, which had an educational motivation to clarify the new classification focused on periodontitis. The strategy used to obtain the articles involved the keywords combined with Boolean operators: "Periodontitis," OR "Periodontal," AND "Classification," AND "Diagnosis," and (NOT "Treatment"). The strategy varied depending on the database, PubMed/MEDLINE, Web of Science, and Embase (Table 1). The focus questions of this review were: (1) "Are all parameters to evaluate periodontitis clearly exposed and explained?"; (2) "Could tooth loss be considered a more important parameter than CAL and RBL to define the severity of the Stage of Periodontitis?"; (3) For the complexity of the case with Periodontitis, are the parameters really well-established to accurately guide the professionals and clinicians to achieve the periodontal diagnosis?

Table 1. Search strategy per database

PubMed/MEDLINE	EMBASE	Web of Science
(("Periodontitis") AND ("Periodontal" OR "Periodontal disease") AND ("Classification") AND ("Diagnosis") NOT ("Treatment"))	#1. (,periodontitis'/exp OR,periodontitis' OR,periodontal disease'/exp OR,periodontal disease') #2. 'classification' #3. 'diagnosis' NOT 'Treatment' #4. #1 AND #2 AND #3 AND [2017-2024]/py	#1. ALL=("Periodontitis" OR "Periodontal Disease") #2. ALL=("Classification") #3. ALL=("Diagnosis" NOT "Treatment") #4. #1 AND #2 AND #3 and 2017 or 2018 or 2019 or 2020 or 2021 or 2022 or 2023 or 2024 (Publication Years)

Eligibility criteria

For inclusion, it was considered all articles published from January 2018 to May 2024 in the English language presenting an educational and instructive approach to the new classification for Periodontitis regarding Stage, specifically, severity and complexity. It excluded any article published that reported only gingivitis or peri-implantitis or had the focus on Grade, or systemic condition correlated to periodontitis; articles that had a primary focus on materials or other substances used in patients diagnosed with periodontitis; populational studies observing the prevalence or incidence of periodontitis; studies evaluating results of professionals and/or students using the new classification; case reports, case series, preprints, chapters, books; any article evaluating periodontal patients who received implant placement; articles that used artificial intelligence (AI) for assessment or development of tools/applications/software; commentaries, opinions, poster in congress, editorial letter or letter to the editor; animal or in vitro studies; and any type of review; same article (duplicated) published in more than one journal.

Study selection

The studies retrieved from the electronic search were screened by two authors (G.F. and J.F.); duplicated studies were excluded. After removing duplicate records, the initial study selection based on title and abstract was performed by the same two assessors who independently screened the articles considering the eligibility criteria. A meeting and discussion resolved disagreements between the two evaluators. The full text of the selected articles and the studies with unclear abstracts was retrieved, and the inclusion in the review was decided by consensus of the two reviewers. Cohen's kappa was performed to evaluate the degree of accuracy and reliability between assessors (inter-agreement level).

Data retrieved

Data collection from the selected studies was performed using a standardized spreadsheet on Excel software (v.16.86, Microsoft Office Excel, 2024). For each included article, the information retrieved included the authors, title, journal name in which the article was published, journal's impact factor (IF), objective, how staging of periodontitis was evaluated, and specific educational details such as flowcharts.

Results

A total of 836 articles were initially found. Three hundred and eighty-eight duplicated articles were excluded. Only 26 articles followed for full-text reading. Then, 6 articles[11,13–17] were included in this critical review. The justification for the exclusions and all screening processes is summarized in Fig. 1. There was a high agreement between the assessors (k = 0.98).

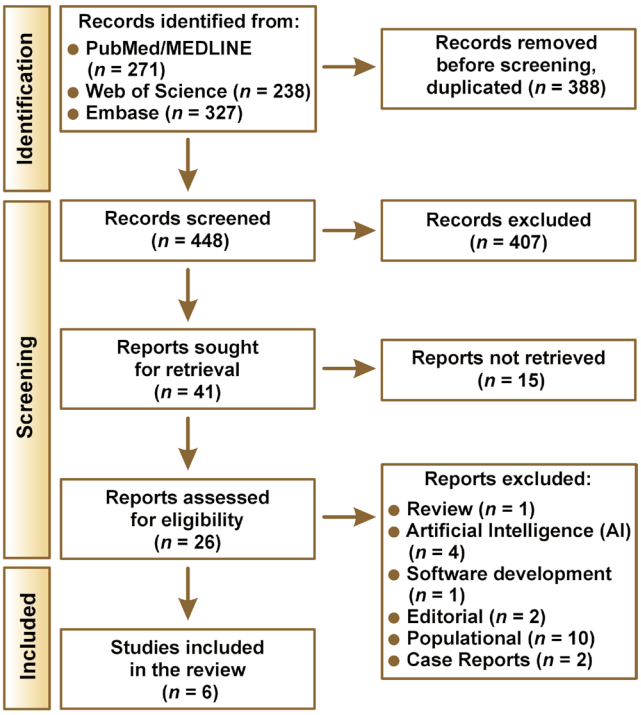

Fig. 1. Flow diagram for the screening and inclusion of articles

In this critical review, 6 articles were included with the presence of 40 authors. The percentual of the authors per country was: Australia (2.4%), China (2.4%), Germany (4.9%), Hong Kong (4.9%), Israel (2.4 %), Italy (2.4%), Spain (4.9%), Switzerland (4.9%), Thailand (4.9%), the Netherlands (2.4%), Turkey (2.4%), the U.K. (24.4%), and the U.S.A. (36.6%). The authors with more participation in the articles included were: Kornman KS (3), Tonetti MS (3), Dietrich T (2), Greenwell H (2), Needleman I (2), Papapanou PN (2), Sanz M (2); the other authors, participated only once.

Current general findings for staging periodontitis based on the new classification system

Due to the measurement error of CA level using a standard periodontal probe and, sometimes, considering the inexperience of the clinician, misclassification of the initial stage of periodontitis is inevitable, thus affecting the diagnostic accuracy.[13] With the disease severity progression, CAL is a more firmly established parameter, permitting the identification of periodontitis with greater accuracy.[13] Then, diagnosing periodontitis initially, prior to staging and grading, should be carried out using the following criteria: the presence of (a) interdental CAL at ≥2 non-adjacent teeth; or (b) buccal/oral CAL ≥3 mm with a probing depth (PD) >3 mm at ≥2 teeth; and (c) the found CAL should not be correlated to non-periodontal causes.[13]

Staging pursues to determine the severity (interdental CAL at the site of greatest loss, %RBL, TLP) and extent (generalized [≥30% teeth involved], localized [<30% teeth involved], molar/incisor pattern) of periodontitis and, then, the complexity of its management (PD, bone loss pattern [horizontal/vertical], furcation involvement, ridge defects, and the need for complex rehabilitation due to masticatory dysfunction, secondary occlusal trauma, bite collapse, drifting, or flaring) based on the amount of periodontitis-induced tissue destruction and specific factors.[13]

Staging can be summarized following the severity and complexity below:

Stage I: a. Severity: CAL ≤1–2 mm, RBL at the coronal third of the root (<15%), and no tooth loss due to periodontitis; b. Complexity: PD ≤4 mm, mostly horizontal bone loss.

Stage II: a. Severity: CAL between 3–4 mm, RBL at the coronal third of the root (between 15% and 33%), and

PERIODONTITIS: STAGING

Sequence of Assessment		Periodontitis	Checklist	Stage I	Stage II	Stage III	Stage IV
1st		Interdental CAL (at site of greatest loss)	☐	1–2 mm	3–4 mm	≥5mm	≥5 mm
2nd		RBL	☐	Coronal third (<15%)	Coronal third (15%–33%)	Extending to middle third of root and	Extending to middle third of root and beyond
3rd For decision between Stage III or IV	Severity	Tooth loss or planned to be extracted (due to periodontitis) Hopeless tooth (mobility class 3)	☐	May have tooth loss		May have no tooth loss or ≤4 teeth	≥5 teeth
4th	Complexity	Local	☐	Maximum PD ≤4 mm (without recession) or PD-GM = 1 or 2 mm	Maximum PD ≤5 mm (without recession) or PD-GM = 3 or 4 mm	AnyPD	AnyPD
5th			☐	Mostly horizontal boneloss: No furcation involvement	Mostly horizontal boneloss: May have Furcation I or II; May have tooth mobility class 1	In addition to Stage II: – May have any class of Furcation involvement – May have vertical bone loss involvement – May have tooth mobility class 1 or 2 – Moderate ridge defects	In addition to Stage III: – May have any class of tooth mobility **Need for complex rehabilitation due to:** – Masticatory dysfunction – Secondary ocelusal trauma (tooth mobility degree ≥2) – Bite collapse, drifting, flaring – May have < 20 remaining teeth (10 opposing pairs) – Severe ridge defects
6th	Extent and distribution	Add to stage as descriptor	☐	For each stage, describe extent as: • Localized (< 30% of teeth involved); or • Generalized (≥ 30% of teeth involved); or • Molar/incisor pattern			

Possibility of change between Stages III and IV

Yellow background = new boxes included; Red letters = modifications; hsCRP = high sensitivity C-reactive protein.
Only complexity factors from Stages III and IV can change the initial Stage obtained through severity factors (red lines and arrows).

Fig. 2. Summarization of the staging proposed by the new classification with suggested modifications (yellow background = new columns and red letters = alterations). It is important to highlight that the sequence recommended must be cumulatively followed. Example 1, Clinical scenario 1: CAL = 4mm; RBL = 20%, 4 teeth loss due to periodontitis; Diagnosis must be kept on Stage II. Example 2, Clinical scenario 2: CAL = 1mm; RBL = 10%, 2 teeth loss due to periodontitis; Diagnosis must be kept on Stage I. It is suggested that the complexity factors should never overcome the severity factors in order to change the Stage. The exception must be considered only for complexity factors between Stages III and IV that can have interchangeability if the initial Stage III or IV was obtained through severity factors (red lines and arrows)

Can a patient's Stage change over time?[14]

(a) Shifting upwards: If a patient has been staged before and had significant disease progression after periodontal therapy, resulting in increased severity and/or more complex treatment needs, then the stage must be shifted upwards at the time of the subsequent examination;

(b) Shifting downwards: Even though the severity of CAL and/or RBL can substantially be reduced after periodontal treatment in cases of successful results or regeneration, the patient is advised to retain the Stage initially assigned. The recommendation for shifting downward cases, remembering that periodontitis is a tooth-dependent disease, is to keep the previous diagnosis for at least 12 months; if the values for CAL, PD, RBL, and GM are improved or are stable after 12 months, a new diagnosis must be performed.

no tooth loss due to periodontitis; b. Complexity: PD ≤5 mm, mostly horizontal bone loss.

Stage III: a. Severity: CAL ≥5 mm, RBL extending to the middle third of root and beyond, and loss of ≤4 teeth due to periodontitis; b. Complexity: PD ≥6 mm, horizontal bone loss, and may have vertical bone loss; may have furcation involvement of class II or III.

Stage IV: a. Severity: CAL ≥5 mm, RBL extending to the middle third of root and beyond, there is the potential for loss of ≥5 teeth due to periodontitis; b. Complexity: PD ≥6 mm, horizontal bone loss and may have vertical bone loss, may have furcation involvement of class II or III, need for complex rehabilitation (masticatory dysfunction, secondary occlusal trauma, bite collapse, drifting, flaring, severe ridge defects, <20 teeth may be present or less than 10 opposing pairs).

Summarization of the included studies

The articles included in this review were deeply analyzed, and all details were included and discussed (Table 2). Figure 2 shows all suggested modifications (for staging). The justifications and explanations for the proposed changes are discussed in the sequence.

Discussion

The new classification system for periodontitis recommends shifting the stage according to whether a stage-shifting complexity factor(s) exists. However, this methodology can create a non-real scenario of a periodontitis case. Then, this critical review strongly suggests a modification for these parameters (complexity); no one of the complexity factors should shift the stage in periodontitis and overcome the severity primarily found. The only exception is for complexity factors from Stages III and IV that can cause interchangeability for the Stages initially obtained through the severity factors (Fig. 2).

In addition, the classification follows that: (a) if any complexity factor(s) is(are) eliminated by the periodontal treatment, the stage should not retrogress to a lower stage since the original stage complexity factor should always be considered in the maintenance phase management.[13] This fact can permit a non-correct scenario analysis of the case. This critical review strongly suggests that if a case has the severity parameters kept stable after 12 months, a new diagnosis must be obtained (shifting downwards); (b) Therefore, if a patient has been staged before and had significant disease progression, even after periodontal therapy, resulting in increased severity and/or more complex treatment needs, in this case, the stage must be shifted upwards at the time of the subsequent examination. This review agrees with this position in order to better treat the case.

Severity: Conflicting parameters

Tooth loss due to periodontitis (TLP) as a parameter to define the stage

It is known that the initial stage of Periodontitis should be determined using CAL (as a result of Periodontitis). If CAL is not immediately available, RBL should be considered. In addition, TLP or a tooth planned to be extracted because of periodontitis currently may modify the stage definition;[13] but in many scenarios, tooth loss information is not tracible or available (without the history of the patient), or it is necessary to trust in the patient's report (there is no accuracy for the information). Hence, to work excellently, the clinician must obtain CAL (developing a new periodontal chart) and RBL and also verify the number of TLP, thus ascertaining the severity. It must be remembered that RBL needs to encompass a substantial portion of the buccal-lingual dimension before conventional radiographs can visualize it; then, the lack of readily discernible RBL does not preclude the presence of periodontitis of incipient severity; this is why the diagnosis of periodontitis is based on CAL rather than RBL.[14] Moreover, the area with CAL must be in 2 non-adjacent sites between 2 teeth to be considered periodontitis.

Tooth loss is currently one of the parameters used to determine the severity of periodontitis. Nonetheless, the impact of tooth loss still needs to be clearly defined in the new classification system. Iwasaki et al.[20] evaluated 374 elderly patients with 7,157 teeth enrolled. The authors registered four lifestyle factors: (1) cigarette smoking, (2) physical activity, (3) relative weight, and (4) dietary quality; scored as healthy (1 point) or unhealthy (0 points) (the least healthy=0; the highest score=4 points). After 6 years, 19.0% of the teeth (n=1,360) exhibited periodontitis incidence or progression, and 8.2% (n=567) were lost. The highest score (4 points) was associated with a significantly lower tooth-specific risk of periodontitis and tooth loss. The authors concluded that simultaneous adherence to multiple healthy lifestyle factors substantially reduces the risk of incidence or progression of periodontitis and tooth loss in older adults. Then, this parameter (TLP) could be better evaluated in the presence of an assessment that includes many other variables that may increase/influence the predictability of periodontal treatment and perspective. This fact shows that around 8% of the teeth were lost after 6 years, which means a low number of TLP and remaining questions about the reliability of using this parameter ("tooth loss").

It is known that there exists a straight relationship between periodontitis and tooth loss. Takedachi et al.[21] evaluated 607 periodontitis patients (mean age of 54.4 ± 11.9 years); 12 (2.0%) had diabetes, 43 (7.1%) were active smokers, and 93 (15.3%) were former smokers, with a mean number of teeth present of 26.1 ± 3.7 at baseline. The total duration (months) of the whole treatment

Table 2. . Details of the articles included regarding Staging for Periodontitis with Criticism and Comments/Suggestions

Authors/ Year	Title/Journal/IF	Objective	Periodontitis Assessment
Maurizio S. Tonetti, Mariano Sanz, 2019 [15]	Implementation of the new classification of periodontal diseases: Decision-making algorithms for clinical practice and education Journal of Clinical Periodontology 8.728	The authors developed empiric decision-making algorithms based on the new classification	- The authors created an extremely interesting flowchart, trying to help clinicians with a faster way to evaluate patients - CAL is the primary criterion for definition of periodontitis (when marginal alveolar bone loss is apparent on diagnostic quality radiographs, it may be an adequate proxy measure of CAL) - PPDs does not allow discrimination of periodontal health, gingivitis, periodontitis, reduced but healthy periodontium, gingival inflammation in a periodontitis patient. Clinicians must recognize the signs of CAL and discriminate them from other clinical conditions also associated with CAL (gingival recession, vertical root fractures, endo-periodontal lesions, loss on the distal of the lower second molars associated with impacted wisdom teeth, or attachment loss secondary to cervical decay or restorations) - Inter-dental CAL in the presence of periodontitis is easier recognized than usually appreciated and requires establishing whether or not the inter-dental CEJ is visible, or the tip of the periodontal probe reaches the root surface in the inter-dental space. - Better explore the reasons for tooth loss with the patient (if it was loose/with mobility or with cavities [caries]) to recognize tooth loss due to periodontitis. Lack of implication of this parameter in case definition and diagnosis leads to the paradox that periodontitis severity may improve as the most compromised teeth are lost.
Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H, Herrera D, Kao RT, Kebschull M, Kinane DF, Kirkwood KL, Kocher T, Kornman KS, Kumar PS, Loos BG, Machtei E, Meng H, Mombelli A, Needleman I, Offenbacher S, Seymour GJ, Teles R, Tonetti MS 2018 [11]	Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions Journal of Periodontology 4.3	This article reviewed, debated and agreed by consensus on the overall conclusions of the five position papers	- Common patterns of CAL were identified across different ages - There is contribution of recession and pocket depth to CAL - Necrotizing periodontal diseases are characterized by three typical clinical features: papilla necrosis, bleeding, and pain; and are associated with host immune response impairments - Endodontic-periodontal lesions are defined by a pathological communication between the pulpal and periodontal tissues at a given tooth, occur in either an acute or a chronic form, and should be classified according to signs and symptoms that have direct impact on their prognosis and treatment; Periodontal abscesses most frequently occur in pre- existing periodontal pockets and should be classified according to their etiology. They are characterized by localized accumulation of pus - Neither age nor sex had any discernible effects on CAL change

Steps for Staging periodontitis	Criticism & Comments
Step 1 a. full-mouth radiographs. Detect marginal bone in any area of the dentition (if available). If bone loss is detectable, the clinician should suspect the presence of periodontitis and move forward to step 2. b. If no radiographs are available or if no bone loss was detectable, it is imperative that the clinician assesses the whole dentition for the presence of signs of inter-dental CAL (presence of visible CEJ or the stopping of the tip of the periodontal probe on the root surface). If inter-dental CAL is detectable, the clinician should suspect the presence of periodontitis. c. If inter-dental CAL is not detected, to evaluate the presence of buccal (oral) recessions with PPDs higher than 3 mm (suspect the presence of periodontitis). d. To ascertain whether CAL is due to local factors only (endo-periodontal lesions, vertical root fractures, presence of caries or restorations or impacted third molars). e. To ascertain whether inter-dental CAL is present in >1 non-adjacent tooth (CAL involves ≥2 non-adjacent teeth, periodontitis) f. If the periodontal charting does not reveal PPD ≥4 mm, then the clinician needs to evaluate the full-mouth BOP (≥10% - gingival Inflammation in a periodontitis patient; <10% - reduced but healthy periodontium). If the periodontal charting shows PPD of 4 mm or more, the diagnosis is a periodontitis case that needs to be further assessed by staging and grading **Step 2** a. Patient is a periodontitis case that needs to be staged: needed full-mouth radiographs, a periodontal chart and a periodontal history of tooth loss (PTL). b. Assess the extent of the disease, by determining whether CAL/BL affects <30% of the teeth (localized) of 30% or more (generalized) c. Define the stage of the disease by assessing severity through CAL, BL, and PTL, and complexity by assessing PPD, furcation and intrabony lesions, tooth hypermobility, secondary occlusal trauma, bite collapse, drifting, flaring or having <10 occluding pairs. **Staging III and IV versus I and II** a. CAL is ≥5mm or BL affects the middle third of the root or beyond, the diagnosis is either stage III or IV periodontitis b. CAL is <5mm, the clinician should look for the presence of class II or III furcation involvement. If present, the diagnosis is either stage III or IV. If no furcation involvement is present, the clinician should check PPD. If PPD is >5 mm, then the diagnosis is either stage III or IV. Clinical judgement should be applied to use PPD to upgrade from Stages I & II to Stage III. **Diagnosis of stage I, II, III or IV** a. Staging for I and II will be based upon the level of CAL and BL. When BL is <15% and CAL is between 1 and 2 mm, the diagnosis is stage I. When BL is between 15% and 33% and CAL is between 3 and 4 mm, the diagnosis is stage II. When BL affects the middle third of the root or beyond and CAL is 5 mm or more, if PTL is 4 teeth or less and in the presence of 10 or more occluding pairs, in the absence of bite collapse, drifting, flaring or a severe ridge defect, then the diagnosis is stage III. When BL affects the middle third of the root or beyond and CAL is 5 mm or more, if PTL is more than 4 teeth and in the absence of 10 occluding pairs, or when existing bite collapse, drifting, flaring or a severe ridge defect, then the diagnosis is stage IV. b. Once the correct periodontitis stage has been determined, the clinician should proceed to determine the grade.	**General** - The flowchart presented was not validated (questions about the diagnostic accuracy and effectiveness) - The article did not clarify or report the best x-ray to check bone loss. Although it is known of all professionals and students, we highlight that is recommend bitewings for measurements and periapical radiographs to evaluate the periodontal ligaments and bone around the root(s). - "Lack of implication of this parameter (PTL) in case definition and diagnosis leads to the paradox that periodontitis severity may improve as the most compromised teeth are lost." - This phrase must be carefully interpreted and cannot be applied for all cases. Periodontitis is a tooth/teeth-dependent condition and if the compromised tooth was extracted and the remaining teeth do not have CAL, cannot justify any treatment for the patient using SRPs (common treatment for periodontitis) **For Step 1** - it seems that the authors want to create a clinical shortcut (due to the high complexity of this classification system) to avoid the complete periodontal evaluation in the beginning (not necessary to do the complete periodontal chart and to have radiographs). The complete periodontal chart and x-rays are mandatory, even though is time consuming, for the adequate evaluation of any periodontal case - evaluation only of the x-ray (if available) without to obtain the periodontal parameters measurements (clinical assessment only if the radiographs are not available). Radiographs are a complementary exam, and the clinical assessment is a mandatory criterion - No evaluation of tooth loss **For Step 2** - full mouth radiographs, periodontal chart, and PTL only if the patient was considered periodontitis case. It could be requested/performed in the beginning for all patients, in order to evaluate all scenario **Staging III and IV versus I and II** - the item b, presented an affirmative condition that cannot be applied for all cases, but most of them. If there is no CAL >5mm (primary criterion of assessment), the complexity factors could not overcome the severity and change the stage, as suggested. The suggestion is to keep the Stage according to CAL, RBL, and PTL, including aggravators (complexity factors) to the Stage found. **Diagnosis of stage I, II, III or IV** - a shortcoming can be observed here for PTL. Stage I and II can present PTL in some cases, e.g., where the extent and distribution are localized molar-incisor pattern. A patient that lost one lower 1st molar and one lower incisor only and have only one 2nd lower adjacent per-molar with CAL = 2mm (mesial and distal); no other teeth with CAL. Again, the suggestion is to keep the Stage based on the severity (CAL and RBL) and present aggravators found in the PTL and complexity
- Loss of periodontal tissue support due to inflammation is the primary feature of periodontitis - A threshold of interproximal, CAL of ≥2 mm or ≥3 mm at ≥2 non-adjacent teeth, is a commonly used parameter - Clinicians typically confirm presence of interproximal tissue loss through radiographic assessments of bone loss - Clinically meaningful descriptions of periodontitis should include the proportion of sites that BOP, and the number and proportion of teeth with probing depth over certain thresholds (commonly ≥4 mm and ≥6mm) and of teeth with CAL of ≥ 3mm and ≥5mm. In the context of clinical care, a patient is a "periodontitis case" if: 1. Interdental CAL is detectable at ≥ 2 non-adjacent teeth, or 2. Buccal or oral CAL ≥3 mm with pocketing ≥3 mm is detectable at ≥2 teeth but the observed CAL cannot be ascribed to non-periodontitis-related causes such as: 1) gingival recession of traumatic origin; 2) dental caries extending in the cervical area of the tooth; 3) the presence of CAL on the distal aspect of a second molar and associated with malposition or extraction of a third molar; 4) an endodontic lesion draining through the marginal periodontium; and 5) the occurrence of a vertical root fracture	"Clinically meaningful descriptions of periodontitis should include the proportion of sites that BOP, and the number and proportion of teeth with probing depth over certain thresholds (commonly ≥4 mm and ≥6mm) and of teeth with CAL of ≥ 3mm and ≥5mm" (a) BOP can be considered in the Periodontal chart but it is not possible to take in consideration to classify periodontitis; this factor may depend on many variables (b) PD and CAL proportions Although these suggestions are excellent, they cannot represent accuracy and will create one more point of debate in the classification. It is suggested more studies on this topic 1. PD is already considered in the complexity of the periodontal classification (periodontitis), and as suggested above, it cannot change the stage found when observed the severity factors (CAL, RBL, PTL)

Authors/ Year	Title/Journal/IF	Objective	Periodontitis Assessment
Maurizio S. Tonetti, Henry Greenwell, Kenneth S. Kornman 2018 [13]	Staging and grading of periodontitis: Framework and proposal of a new classification and case definition Journal of Periodontology 4.3	To review evidence and rationale for a revision of the current classification, to provide a framework for case definition that fully implicates state-of-the-art knowledge and can be adapted as new evidence emerges, and to suggest a case definition system that can be implemented in clinical practice, research and epidemiologic surveillance	A patient is a periodontitis case in the context of clinical care if: 1. Interdental CAL is detectable at ≥ 2 non-adjacent teeth, or 2. Buccal or oral CAL ≥3 mm with pocketing >3 mm is detectable at ≥2 teeth and the observed CAL cannot be ascribed to non-periodontal causes such as: 1) gingival recession of traumatic origin; 2) dental caries extending in the cervical area of the tooth; 3) the presence of CAL on the distal aspect of a second molar and associated with malposition or extraction of a third molar, 4) an endodontic lesion draining through the marginal periodontium; and 5) the occurrence of a vertical root fracture. - BOP itself, or as a secondary parameter with CAL, does not change the initial case definition as defined by CAL or change the classification of periodontitis severity - The severity score is primarily based on interdental CAL in recognition of low specificity of both pocketing and marginal bone loss, although marginal bone loss is also included as an additional descriptor (based on the worst affected tooth in the dentition). Only attachment loss attributable to periodontitis is used for the score - The complexity score is based on the local treatment complexity assuming the wish/need to eliminate local factors and takes into account factors (vertical defects, furcation involvement, tooth hypermobility, drifting and/or flaring of teeth, tooth loss, ridge deficiency and loss of masticatory function) - Besides the local complexity, it is recognized that individual case management may be complicated by medical factors or comorbidities - CAL to determine the initial stage in the severity dimension. Some clinicians may prefer to use diagnostic quality radiographic imaging as an indirect and somehow less sensitive assessment of periodontal breakdown. This may be all that is necessary to establish the stage.
Kenneth S. Kornman, Panos N. Papapanou 2020 [14]	Clinical application of the new classification of periodontal diseases: Ground rules, clarifications and "gray zones" Journal of Periodontology 4.3	Reiterate some basic principles, emphasize important "ground rules," identify potential gray zones, and provide practical tips that will help clinicians to seamlessly navigate the new system in their everyday clinical practice	- Stage reflects the severity of the disease (expressed through CAL and RBL), but also tooth loss that has occurred as a result of periodontitis, at least as well as can be determined. it reflects anticipated complexity of treatment required to eradicate/reduce the current level of infection and inflammation - Grade describes additional biological dimensions of the disease including the observed or inferred progression rate, the risk for further deterioration due to environmental exposures (smoking) and co-morbidities (diabetes), and the risk that the disease or its treatment may adversely affect the patient's general health status - BOP is a valuable clinical parameter to help assess current levels of inflammation and residual risk post-treatment, but BOP does not influence the classification

Steps for Staging periodontitis	Criticism & Comments
Severity - Stage I = Initial periodontitis; Stage II = Moderate periodontitis; Stage III = Severe periodontitis with potential for additional tooth loss; Stage IV = Advanced periodontitis with extensive tooth loss and potential for loss of dentition - CAL and RBL will be the primary stage determinants - If a stage shifting complexity factor(s) were eliminated by treatment, the stage should not retrogress to a lower stage since the original stage complexity factor should always be considered in maintenance phase management. A notable exception is successful periodontal regeneration that may, through improvement of tooth support, effectively improve CAL and RBL of the specific tooth - Conventional definitions of severe periodontitis need to be revised to better discriminate the more severe forms of periodontitis - Another important limitation of current definitions of severe periodontitis is a paradox: whenever the worst affected teeth in the dentition are lost, severity may actually decrease. Tooth loss attributable to periodontitis was incorporated in the definition of severity **Complexity of management** - Factors (PD, type of bone loss (vertical and/or horizontal), furcation status, tooth mobility, missing teeth, bite collapse, and residual ridge defect size) increase treatment complexity and need to be considered and should ultimately influence diagnostic classification. Explicit designation of case complexity factors helps to define levels of competence and experience that a case is likely to require for optimal outcomes	**Severity** - "whenever the worst affected teeth in the dentition are lost, severity may actually decrease. Tooth loss attributable to periodontitis was incorporated in the definition of severity." A shortcoming can be observed for the phrase above; Stage I and II, that suggested no tooth loss, can present PTL in some cases, e.g., where the extent and distribution are localized molar-incisor pattern. A patient that lost one lower 1st molar and one lower incisor only and have only one 2nd lower adjacent per-molar with CAL = 2mm (mesial and distal); no other teeth with CAL. The suggestion is to keep the Stage based on the severity (CAL and RBL) and present aggravators found in the PTL and complexity. **Complexity of management** - Even with all factors that can be present in the complexity of a case, it is suggested that all of them cannot modify the initial diagnosis of periodontitis found with the evaluation of CAL and RBL. Using PTL and the complexity factors, it is possible to change between Stage III and IV only **Examples described in the article** (1) "in case of very short common root trunk with a CAL of 4 mm, which may have resulted in class II furcation involvement; the classification recommended was Stage II; hence shifting the diagnosis from stage II to stage III periodontitis" (2) In the same case above, "if posterior bite collapse is present then the stage IV would be the appropriate stage diagnosis since the complexity is on the stage IV level" Once again, it is suggested to initially determine stage of periodontitis, must be used CAL and RBL; the other parameters should be considered and registered but they cannot change the diagnosis (Stage). The cases above were presented without a good contextualization of them
1st step – Periodontitis a. CAL: if (1) interproximal CAL is present at least at two different, non-adjacent teeth, and (2) the observed CAL cannot be attributed to traumatic factors or non-periodontitis related etiologies (e.g., root fracture, endodontic infection, surgical trauma) b. In the absence of interproximal CAL, but if CAL that cannot be ascribed to non-periodontitis-related causes is present at buccal or lingual surfaces, a diagnosis of periodontitis requires concomitant presence of CAL of ≥3 mm and PD of ≥3 mm at ≥2 teeth c. Confirm the presence of CAL by corresponding interproximal RBL. Do not use of RBL as the primary criterion (under-detection of incipient periodontitis and an increase in "false negatives") **2nd step – Stage (severity)** - Stages I and II in adult patients (incipient or moderate severity, no loss of any teeth) are likely very different from Stages III and IV (one or several intrinsic or environmental risk factors, more complex cases) - Staging: medical history, radiographs, and probing chart to distinguish between Stage I or II versus Stage III or IV periodontitis (severity of tissue damage and the presence of periodontitis-associated tooth loss) – to study in detail the available full-mouth periodontal charting and full-mouth series of intra-oral radiographs - RBL: bone loss of up to 15%; extending between 15% and 33% of the root length (not necessary a high level of precision) and extending to middle third of root and beyond. The intention is to distinguish between an incipient stage from more substantial bone loss - If in the assessment the patient was classified as Stage III (severe periodontitis) or Stage IV (very severe periodontitis) periodontitis, PTL can be attributed to periodontitis (one to four teeth versus five or more teeth lost); or then, on the presence of the various complexity factors. It must be realized that either Stage III or Stage IV **Step 3** - Complexity: e.g., Stage IV - periodontitis threatens the entire dentition and, consequently, treatment requires extensive oral rehabilitation **Step 4** - Extent and Distribution: "localized" or "generalized" describe the extent of the dentition that is affected by the Stage-defining severity - Can a patient's Stage change over time? (a) If a patient that has been staged at a given time point experiences significant disease progression or disease recurrence after therapy that results in increased severity and/or more complex treatment needs, then stage must be shifted upwards at the time of the subsequent examination, as appropriate (b) However, although the severity of CAL and/or RBL can be reduced substantially from beyond the coronal third to within the coronal third in cases of successful regeneration therapy, it is advised that the patient retains the Stage originally assigned prior to the treatment	**1st step – Periodontitis** - It is suggested that PTL can be present in Stage I and II; but primarily, it is necessary to obtain the CAL and RBL for the correct diagnosis - "BOP is a valuable clinical parameter to help assess current levels of inflammation and residual risk post-treatment, but BOP does not influence the classification" – BOP cannot have any influence on the diagnosis of periodontitis - "Confirm the presence of CAL by corresponding interproximal RBL. Do not use of RBL as the primary criterion (under-detection of incipient periodontitis and an increase in "false negatives")" – always to use CAL as 1st criterion of diagnosis **2nd step – Stage (severity)** - It is suggested to consider, even though cannot be so common, the existence of tooth loss in Stages I and II **Step 4** - "In cases of successful periodontal regeneration therapy, it is advised that the patient retains the Stage originally assigned prior to the treatment" – It is suggested to keep the previous diagnosis for at least 12 months; if the values (numbers) are kept improved/stable, a new assessment and diagnosis must be performed

Authors/ Year	Title/Journal/IF	Objective	Periodontitis Assessment
Pimchanok Sutthiboonyapan, Hom-Lay Wang, Orawan Charatkulangkun 2020 [16]	Flowcharts for Easy Periodontal Diagnosis Based on the 2018 New Periodontal Classification Clinical Advances in Periodontics 0.7	Flowchart designed for quick initial screening to make proper diagnosis for three most commonly found periodontal conditions (health, gingivitis, and periodontitis) to differentiate the types of periodontitis diagnosis by using staging and grading system	- The authors developed an interesting flowchart. It can be extremely useful in order to accelerate the periodontal assessment - Otherwise, there is an inconsistence in the severity of periodontitis analysis: 1st parameter used was "tooth loss" due periodontitis instead of CAL and RBL - Grade
T. Dietrich, P. Ower, M. Tank, N. X. West, C. Walter, I. Needleman, F. J. Hughes, R. Wadia, M. R. Milward, P. J. Hodge, I. L. C. Chapple 2019 [17]	Periodontal diagnosis in the context of the 2017 classification system of periodontal diseases and conditions – implementation in clinical practice British Dental Journal 1.626	Periodontal diagnosis in the context of the 2017 classification system of periodontal diseases and conditions – implementation in clinical practice	- To create an algorithm for clinical periodontal assessment of plaque-induced periodontal disease - The authors proposed a flowchart mixing BPE with the new classification system

Clinical attachment loss (CAL), Bone loss (BL), Probing pocket depths (PPDs), Probing depth (PD), Cement-enamel junction (CEJ), Periodontal history of tooth loss (PTL), Scaling and root planing (SPR), Bleeding on probing (BOP), Radiographic bone loss (RBL), Basic Periodontal Examination (BPE). Red letters = suggestions for changes and improvement.

Steps for Staging periodontitis	Criticism & Comments
Severity of periodontitis 1. tooth loss from periodontitis, including teeth planned for extraction due to periodontitis. If tooth loss existed, then the case is either stage III or IV 2. The differentiation of stage III or IV is based on the number of teeth lost and masticatory dysfunction (a) ≥5 teeth and/or <20 remaining teeth and/or need a rehabilitation because of masticatory dysfunction, periodontitis stage IV (b) ≤4 teeth lost due to periodontitis and no other masticatory dysfunction, then stage III (c) no tooth loss or has tooth loss from reasons other than periodontitis or unknown cause, a combination of CAL, PD, and RBL will be used to classify the patient 3. Then, (a)CAL ≥5 mm and/or PD ≥6 mm and/or vertical bone loss ≥3 mm and/or furcation involvement grade 2 or 3, the case is either stage III or IV (masticatory dysfunction and/or number of the remaining teeth will then be used to determine the stage) (b) CAL is <5 mm and/or PD <6 mm, stage I or II is assigned **Grade of periodontitis** 4. Grade B is usually the default for most periodontitis cases and a clinician will consider if it should be adjusted to grade A or grade C (a) Primary criteria for grade: disease progression - direct evidence from longitudinal data (>5 years) of RBL or CAL, or the indirect evidence from a calculation of percentage of bone loss per age (b) Pattern of periodontal destruction. If there is evidence of rapid progression or inconsistency of biofilm and periodontal destruction - grade C. However, if there is no evidence of periodontal disease progression or percentage of bone loss per age <0.25, grade A is assigned. The presence or control of risk factors can also modify the grade assignments. For example, if the patient is a heavy smoker or has uncontrolled diabetes, periodontitis grade B can be modified to grade C	**Severity of periodontitis** - The first point of analysis for staging periodontitis in this flowchart was tooth lost or planned to be extracted. This fact is going against the original classification and many mistakes can be found in this stage. It is highly recommended avoiding to use tooth loss as the first parameter; moreover, it is suggested CAL and RBL to be analyzed before tooth loss - CAL and RBL were used as secondary parameters; this fact is contrary of the proposal of the new classification and can invalidate the correct use of the flowchart related to the new classification - although this flowchart seems extremely useful, it is making mistake to find the Stage of periodontitis. In a clinical trial developed by the authors to validate this flowchart, they recognized that "Modifications of the proposed flowcharts could enhance the accuracy of the periodontal diagnosis. Most errors in the full diagnosis were in the details of each diagnosis rather than disease identification, especially in periodontitis cases". - The authors affirmed: (a) "This implied that the flowcharts for periodontitis stage and grade provide accuracy for identifying periodontitis extent and grade comparable to the consensus reports (75.93 vs. 72.39%, respectively), and provide better accuracy of the assigned periodontitis stage" (b) "For identifying periodontitis cases, the group using flowcharts obtained higher median scores than the group using consensus reports (p=0.004)" - Therefore, the authors are trying to cause a confusion on the consensus performed, deciding by themselves that evaluate tooth loss before CAL and RBL will lead the clinicians to a better result than the decision obtained of all experts in the consensus. Again, the idea of evaluating first tooth loss is not the original commandment of the new classification, which must be taken in consideration
The article posed a lot of true questions for staging: (a) "The BSP implementation group recognized several challenges with the proposed periodontitis staging grid for implementation in general dental practice, specifically: • The lack of an unambiguous decision rule that describes how the various parameters in the staging grid should be combined to determine a patient's disease stage • The fact that clinical attachment loss is not routinely measured in clinical practice • The inclusion of complexity measures such as tooth loss due to periodontitis and alveolar ridge defects, which may be difficult to ascertain and/or may not be well defined." Although all these points are correctly posted, the clinician must include in the daily routine not only the use of PD, BOP, and tooth loss, but also CAl and GM position, in order to work adequately (even it increases the time of the appointment) - BPE is divided in 4 codes: '0' indicates that no treatment is required '1' and '2' mean that a basic clean is needed '3' and '4' means gum disease is advancing and subsequently requires advanced therapy - if PPD is at least 4 mm (BPE code 3); if the PPD is at least 6 mm (BPE code 4) - staging and grading from the new classification were summarized/adapted for periodontitis cases: (a) interproximal bone loss (Staging): <15% or RBL <2 mm (Stage I); Coronal third of root (Stage II); Mid third of root (Stage III), and Apical third of root (Stage IV) (b) % bone loss / age (Grade): < 0.5 (Grade A); 0.5-1.0 (Grade B); > 1.0 (Grade C) - Extent and distribution were equal of the new classification	- The flowchart proposed a mix of the Performing a BPE entails 'walking' the probe around each tooth and recording only the worst score (code 0–4) in each sextant for efficiency - The authors explained that BPE and its equivalent systems have been well established in the clinical community across Europe due to its relative simplicity and efficiency. Then, they modified the BPE original version, mixing with part of the new classification, but adapted - the initial evaluation using BPE is based on the recession in the interdental area, BOP and PPD; then, it is unable to identify patients with historical periodontitis; this system does not use CAL or RBL - The use of BPE on a periodontal patient (with periodontitis) and no BPE scores greater than 2, would wrongly result in a provisional classification of periodontal health (<10% sites with BOP), localized gingivitis (10–30% sites with BOP) or generalized gingivitis (>30% sites with BOP), rather than capture the fact that the patient is a periodontitis patient with a current status of health or gingival inflammation "As per current BSP guidance a maximum BPE code of 3 would trigger a panoramic radiograph and/or selective periapical radiographs, which will allow determination of percentage bone loss relative to the root length." They suggested the use of radiographs that are not the best to evaluate measures. Therefore, in the Stage section, they suggested bitewings for the posterior areas Summarizing, the article introduced a new methodology/adaptation for periodontal evaluation which abandoned/unsettin part of the new classification. Moreover, this algorithm must be validated

period, active periodontal therapy (APT) period, and supportive periodontal therapy (SPT) period was, respectively, 80.9 ± 34.2 (range: 16 to 190 months), 11.1 ± 6.4 (range: 2 to 35 months), and 69.9 ± 35.3 (range: 12 to 174 months). 176 patients (29.0%) were classified into stage III grade C, followed by 159 (26.2%) in stage III grade B, and 128 (21.1%) in stage II grade B. During the treatment period, 260 teeth (63 during APT and 197 during SPT) out of 15,838 were lost (1.64%). They reported that patients in stages I and II (grades A, B, or C) had no TLP during the treatment period. Patients in stage IV and grade C had TLP rates of 0.24±0.31 and 0.15±0.24 (number of teeth/patient/year), respectively, with significant differences from those in the other stages and grades. TLP rates were higher in stage IV and/or grade C patients during both APT and SPT. Multivariate analysis revealed that stage IV and grade C as independent variables were significantly associated with the number of instances of TLP not only during the total treatment period and also during APT or SPT. The results of this study suggested that the new classification has a significantly strong association with TLP during both APT and SPT and that patients diagnosed with stage IV and/or grade C periodontitis had a higher risk of TLP during both periods. Thus, it was possible to observe that TLP was totally correlated to stages III and IV; this fact led us to understand that this parameter is highly important only in deciding the severity of those stages (III or IV) (Fig. 2). Moreover, if the patient is qualified for Stage III or IV (CAL ≥5mm and RBL extending to the middle third of the root and beyond), therefore, without any TLP or tooth due to another reason, the patient must be framed in Stage III. This critical review suggests that "May have no tooth loss" for Stage III (Fig. 2).

Even without precision regarding the impact of TLP on periodontitis, some authors[16] reported that this parameter should be considered the most important in defining the severity of periodontitis, compared to CAL and RBL. They considered tooth loss the first criterion of analysis for staging Periodontitis, ignoring the new classification recommended as primary criteria CAL and RBL. It was described in their article, "In the flowchart for periodontal stage, information of TLP was selected as the first criteria to separate patients with severe periodontal conditions, which can be stage III or IV". Even though it can be a good strategy and a shortcut to sift patients, it can lead to mistakes. Also, the authors included that "in the case that periodontitis is diagnosed from the flowchart but with no obvious RBL/CAL, clinicians must confirm the diagnosis again, considering the periodontitis case definition"; hence, without obvious CAL/RBL (which were not measured), it is necessary to redo the periodontal assessment.

Then, a question is posed: What is the reliability of this criterion (TLP) as the first evaluation parameter? In some cases, the patient needs to be informed of the reason for the extraction because the professional does not have a history of previous treatments. Moreover, the authors affirm this criterion is enough to find patients with severe periodontitis; again, it can be an interesting strategy; nevertheless, it does not follow the concepts proposed by the new classification system, which recommended CAL and RBL for the initial assessment and can generate a non-precise result.

Therefore, screening patients considering TLP as the primary deciding factor for staging the severity of Periodontitis is not suggested. This information may lead clinicians to misunderstand, misinterpret, and possibly make mistakes in finding the severity and stage, which are extremely necessary to define the periodontal diagnosis and treatment plan. Moreover, due to this type of approach worldwide, many educators, students, and professionals are using the concept of "TLP" as the primary criterion for the severity of periodontitis, completely disregarding CAL and RBL. Additionally, this approach completely overlooks specific cases that can justify its non-application, such as teeth loss posed by the former localized aggressive periodontitis (periodontal disease with rapid progression) (example 1) or in the case of complete maxillary teeth extractions for rehabilitation without a periodontal reason, resulting in less than 10 opposing teeth pairs (example 2).

Example 1: Latin male patient (22 years old) with 26 remaining teeth (without wisdom teeth). Only one of them, 2nd premolar [ADA #20 or FDI #35], with CAL in the mesial (PD = 5mm; CAL = 2mm) and distal (PD = 5mm; CAL = 2mm); the other two teeth were lost due to periodontitis (central incisor [ADA #9 or FDI #21] and 1st molar [ADA #19 or FDI #36]), without any other tooth being affected by periodontal issues. Observing the current scenario, with 2 teeth lost to periodontitis and only 1 remaining tooth with periodontitis, the patient was diagnosed with Localized Periodontitis Stage III (former Localized Aggressive Periodontitis – a periodontal disease with rapid progression). Therefore, it does not make sense to consider Stage III because of the number of teeth lost, not considering the CAL and RBL. In addition, typically, periodontitis is treated with scaling and root planning (SRP) procedure; however, where could it be used to treat the case demonstrated in example 1? Possibly only on tooth #20/#35. This fact (Stage III) does not agree with the actual severity of the periodontal disease found, recommending a more accurate diagnosis, resulting in Localized Periodontitis Stage I, after ascertaining the CAL.

Example 2: Patient, female (50 years old) with a long history of caries and periodontal disease. She arrived for evaluation with an edentulous maxilla without any wisdom teeth and 11 lower remnant teeth. Two of them, posteriors, were planned to be extracted due to decay. The PD found was 2-3mm for all the present teeth with a general GM of 1mm (normal position [1mm coronally CEJ]); no CAL or RBL was observed. Then, the clinical assessment resulted in less than 10 opposing teeth pairs

and undefinition for the reason of other extractions. If followed the suggestion of the flowchart above, this fact led to the direct diagnosis of Periodontitis Stage IV (<10 opposing pairs and complex rehabilitation). Therefore, to treat the most severe level of periodontitis (Stage IV), it is usually necessary to make appointments for SRP. But where should we apply SRP in this case? It does not fit for this scenario. Observing the remaining teeth, this case could be considered Periodontal Health or, depending on the BOP result, gingivitis, based on CAL and RBL present (without previous history).

The suggestion of this critical review is to remove "no tooth loss due to periodontitis" from the official recommendation for Stages I and II, which may have or may not have TLP, and keep this parameter only for differentiation between Stages III (it may not have TLP) and IV (Fig. 2), but after assessing CAL and RBL. This fact will permit the clinicians not to consider first TLP (involving periodontally hopeless tooth, which means irrational to treat - where the CAL approximates the apex of the root circumferentially, in combination with a high degree of tooth hypermobility, degree III),[22] reaching a more accurate diagnosis. Furthermore, remembering that the 1st and 2nd analysis parameters are CAL and RBL, which depend on the PD and GM position is worth remembering. All of them must be acquired before evaluating the number of tooth losses to define the stage of periodontitis.

Thus, summarizing the assessment of severity for periodontitis, this critical review suggests and strongly recommends checking the parameters cumulatively, following the sequence: 1st – CAL (with PD and GM); 2nd – RBL; 3rd – tooth loss (for decision between Stages III and IV) (Fig. 2). It is also important to highlight that if the patient has TLP (3 teeth), but the worst site CAL is 4mm, the Stage must be kept on Stage II, respecting the cumulative sequence suggested (Fig. 2).

Complexity

In the new classification system,[13] the authors recommended: "Complexity factors may shift the stage to a higher level". Besides CAL, RBL, or TLP (severity factors), the role and relative importance of the complexity factors of periodontitis in defining the stage cannot be justified only by PDs, furcation status, tooth mobility, type of bone loss, the extent of ridge defect, masticatory dysfunction, and missing teeth or a number of opposing pairs as proposed by the classification. Thus, this review strongly suggests an adjustment for the above affirmation that considers the complexity factors sometimes more relevant than the severity factors. The suggestion for modification is that never one complexity parameter can overcome a severity parameter, changing the initial Stage obtained following CAL (1st), RBL (2nd), and TLP (3rd). An exception must be respected only for complexity factors between Stages III and IV that can change the initial Stage III or IV obtained, only between themselves, according to the complexity found in the case (Fig. 2).

Furcation

The mean root trunk lengths (RTL) reported when vertically assessed (from cementoenamel junction [CEJ] to furcation) in maxillary and mandibular molars is 4.31 mm (minimum of 3mm and maximum of 8 mm).[23] This result helps clinicians to find better decision-making during the management of periodontal disease conditions. Therefore, in some cases where RTL is extremely short (CEJ-furcation=3mm), if there were a CAL of 3mm in the buccal area of a molar (#30 ADA or #46 FDI) with PD of 5mm in this face, for example, it would reach and compromise the furcation area.

Thus, analyzing the case above (CAL of 3mm in the buccal area of a molar with PD of 5mm in the same surface, with Furcation class II involvement, and only two more non-adjacent teeth with the interproximal bone loss [CAL = 1mm and PD = 4mm]): what would be the correct diagnosis of this case ([a] Stage II because of the higher CAL [2mm] with PD=5mm in the buccal area; or [b] Stage III because of the Furcation class II without a 6mm of PD)? This critical review suggests that complexity never should overcome the severity; then, the result for the case above is (a) Stage II (CAL=3mm with PD=5mm, with Furcation class II involvement). Thereby, this article suggests a modification for the Furcation involvement, as follows: Stage I (no Furcation involvement); Stage II (may have Furcation class I or II involvement); and Stages III and IV (may have or not have any Furcation involvement). Previously, the Furcation classes II and III were only considered in Stages III and IV, and Furcation I was not included.

In another similar case, CAL of 4mm in the buccal area of a molar with PD of 6mm in this surface, with Furcation class I involvement, and only two non-adjacent teeth with interproximal bone loss (CAL=1mm and PD=4mm): what would be the correct diagnosis? Again, following the suggestion of this critical review, the complexity factors should never overcome the severity factors; then, the result for this new case is Stage II (CAL=4mm). This review suggests including Furcation class I involvement in Stage II of periodontitis. Even though there was PD=6mm in this case, it should not overcome the severity found.

Probing depth (PD)

Keeping PD as the primary initial clinical criterion is a good clinical option because it can be easily obtained.[16] PD can indicate the presence of an active periodontal-diseased pocket,[24] and deep pockets have a higher risk of disease progression than shallower pockets.[25] Therefore, at the same time or appointment that the clinician is analyzing the PD, it is highly recommended to evaluate gingival

margin position (GM) and CAL in order to be less time-consuming, which also depends on the level of experience of the professional and assistant. To correctly work in Periodontics, CAL must be obtained (the most important parameter); PD can be a primary evaluation factor but not to define periodontitis diagnosis.

As a "tip" or suggestion to quickly find CAL (which must be confirmed with the RBL analysis), it is possible to calculate it as demonstrated in Table 3. It is important to understand that it is a formula to quickly calculate the CA level (typically, the "normal" position of the GM is (+)1mm above CEJ; but it is possible to find (+)2mm or sometimes more); in order to have higher accuracy, the position of the GM must be clinically measured detecting the CEJ and real position of GM ([+], above CEJ; [-], below CEJ).

Some scenarios bring much confusion for periodontitis diagnosis (staging) if it follows the initial evaluation using PD. The metric typically accepted as a normal PD is up to 3mm. Therefore, observing the PD considered for the complexity of a case, 4mm is an adequate metric for Stage I, which is well-registered in the new classification. This article suggests that PD=4mm must be without recession involvement or, if the recession is present, to do the simple calculation presented above (PD – GM [the result must be around 1 and 2]). It is necessary to remember that any GM value must be positive if coronal to CEJ, zero "0" if GM=CEJ, and negative if there is any recession.

Similarly, it can be observed that the PD proposed in Stage II was ≤ 5mm; it must be kept. This review suggests only adding without recession involvement or doing the simple calculation PD – GM, which should result between 3 and 4 if the recession is present.

To avoid creating questions for Stages III and IV, which initially considered the necessity of finding PD ≥6mm during the clinical evaluation, this critical review suggested considering the presence of any PD for Stages III and IV. This suggestion is based on the possibility of a case with multiple CAL ≥5mm with generalized recession and PD lower than 6mm for all teeth. Again, it is worth remembering that this review suggests that complexity factors should not overcome the severity factors, except between Stages III and IV.

Mobility

This parameter was not directly considered among the complexity factors, but this critical review suggests including it in Stage II (mobility 1), Stage III (mobility 1 and 2), and IV (any mobility). A tooth with mobility 3 is considered hopeless, and even though it was considered in Stage IV, it is most adequate as a hopeless tooth (TLP).

Bite collapse, drifting and flaring

There is confusion in the literature about using this parameter. The initial idea of the presence of this content (bite collapse, drifting, and flaring) is strictly associated with the absence of teeth (≥5 teeth), which caused a need for complex rehabilitation. Suppose patients have no tooth loss or TLP of ≤4 teeth (without a need for complex rehabilitation) presenting any type of drifting or flaring, with CAL ≥5mm and RBL extending to the middle third of the root and beyond. In that case, it cannot be a justification for changing the diagnosis from Stage III to Stage IV.

Again, this critical review suggests that no one parameter from complexity must overcome severity parameters; an exception must be respected only for complexity factors between Stages III and IV that can change the initial

Table 3. Simplified strategy to faster calculate clinical attachment (CA) level

CONDITION	FORMULA (CA level = PD – GM)
a. Tooth without recession: Without recession means that it is considering the GM in the "normal" position (+1mm above CEJ) – It is suggested GM (+)1mm coronally to CEJ to facilitate the calculation; therefore, normally, this number can be greater (check clinically this measure from GM to CEJ for greater accuracy) – It is necessary to remember and consider +2mm of the supracrestal tissue attachment (former biological width)	**PD = 4mm; GM = +1mm** CA level = 4-1 = 3mm; (remember that 2mm belongs to the biological width); there is no CAL or, then, 1mm of CAL (needs a deeper assessment of the case) **PD = 5mm; GM = +1mm** CA level = 5-1 = 4mm (-2mm biological width) = 2mm of CAL **PD = 6mm; GM = +1mm** CA level = 6-1 = 5mm (-2mm biological width) = 3mm of CAL
b. Tooth with recession: (GM at the same level CEJ [CEJ = GM] or apically positioned)	**PD = 2mm; GM = 0 (buccal recession)** CA level = 2mm - 0 = 2mm (No CAL) – it is not Periodontitis (needs of a deeper assessment of the case) **PD = 3mm; GM = 0 (buccal recession)** CA level = 3mm - 0 = 3mm (CAL = 3mm) **PD = 4mm; GM = 0 (interdental recession)** CA level = 4-0 = 4mm of CAL **PD = 2mm; GM = -1 (interdental recession)** CA level = 2-(-1) = 3mm CAL **PD = 2mm; GM = -2 (interdental recession)** CA level = 2-(-2) = 4mm CAL

CA level = clinical attachment level; CAL = clinical attachment loss; PD = probing depth; CEJ = cementoenamel junction; GM = gingival margin (from CEJ to GM); mm = millimeters.

Stage III or IV obtained, only between themselves, according to the complexity found in the case (Fig. 2); however, for the case above, there is no justification to consider it.

'Gray zones' for staging periodontitis

Ravidà et al.,[26] Abrahamian et al.,[27] and Gandhi et al.[28] agree that more efforts are needed to improve diagnostic agreement among professionals, especially general dentists, for the case definition of periodontitis. Their studies identified "gray zones" using the new classification system, which must be revised and clarified; they can result from the experts' non-concordant opinions and diagnoses. Typically, most of them involve conflicting severity and complexity factors among Stages III and IV.

One of the gray areas to discuss in this critical review is "tooth loss due to periodontitis" (TLP). The new classification acknowledges TLP as part of the severity of staging periodontitis. Therefore, if the professional has no longitudinal patient data available to support the missing tooth allocation as TLP, the patient will be the source of information. The literature suggests easy ways to obtain it, such as asking about tooth mobility or cavities (correlated symptoms). If history cannot be provided, the tooth loss cannot be considered TLP. However, what is the reliability of this information, if available, to help diagnose the case? As discussed above, this parameter is important, but it is suggested that it cannot be more relevant than CAL and RBL. Thus, this critical review suggests modification that the severity should be obtained following and respecting the cumulative sequence of CAL (1st), RBL (2nd), and TLP (3rd). The TLP may be present or not in Stages I, II, and III; therefore, it can be a factor of differentiation between Stages III and IV.

Another "gray zone" point to discuss is whether complexity factors can shift a patient's severity level. Before, as clearly reported in the new classification,[26] shifting upwards can be performed if a patient has been staged before and had significant disease progression after periodontal therapy, resulting in increased severity and/or more complex treatment needs. Then, the stage must be shifted upwards during the subsequent examination.[14] Otherwise, for shifting downwards, even though the severity of CAL and/or RBL can substantially be reduced after periodontal treatment in cases of successful results or regeneration, the patient is advised to retain the Stage initially assigned. The recommendation of this critical review for shifting upwards is keeping the same concept adopted by the new classification; whereas shifting downward is based on the fact that periodontitis is a tooth-dependent disease, and if the patient holds the previous diagnosis for at least 12 months, with all values for CAL, PD, RBL, and GM improved or stable within that period (12 months), a new diagnosis must be performed.

Returning to the question above (complexity factors can shift a patient's severity level), stage IV periodontitis has many parameters to be evaluated in complexity (masticatory dysfunction, secondary occlusal trauma, bite collapse, drifting, flaring, severe ridge defects, less than 10 opposing pairs), besides CAL, RBL, and TLP (≥5 teeth), which is different from Stage III, needing for multidisciplinary rehabilitation. In contrast with Stage III, which also presents severe periodontal tissue support loss, Stage IV periodontitis involves a larger segment of the dentition. Thus, stage I or II periodontitis cases can never be upshifted to Stage IV directly based on the complexity factors alone because of the number of complexity factors involved (it is necessary to observe the severity factors, too). Some examples that are classified directly as Stage IV by mistake involve (a) partially edentulous cases with <10 opposing pairs, where tooth loss is due to reasons other than periodontitis (primary occlusal trauma, with loss of vertical dimension of occlusion or tooth drifting); (b) patients who present with all posterior teeth lost due to unknown reason, and the clinician infers the justification based on the oral and general health history and assessment of the current periodontal status.[26] In order to find a simple solution, this critical review suggests that any complexity factors found should never overcome the severity factors to change the Stage. If this parameter is followed, many mistakes in diagnosis will be avoided. The exception for this parameter suggested must be considered only for complexity factors between Stages III and IV; they can have interchangeability if the initial Stage III or IV was obtained through severity factors.

Reassessing clinical cases with the 'gray zones' published in the literature using the new suggestions for staging periodontitis

This part includes three articles that published cases reporting "gray zones" for periodontitis; they must be included and discussed because of their importance in the literature. The cases were presented with the original result found (left) and suggested modification (right).

1. Sirinirund et al.[29] reported 2 cases with "gray zones" for periodontitis. Both cases had generalized periodontitis.

(a) Case 1 was a 46-year-old Caucasian female, former smoker (10 cigarettes/day for 5 years and quit for more than 20 years), with uncontrolled type 2 diabetes mellitus (HbA1c=9.4%) and morbid obesity (body mass index=50.6 kg/m^2); patient had deep overbite along with tooth drifting/flaring in the upper anterior of the maxilla, without substantial loss of vertical dimension, mobility, or masticatory dysfunction; the patient had no missing teeth. The greatest CAL and PD found was 11mm (#5 ADA / #14 FDI), with GM=0, RBL to mid-third of root length or beyond, with a history of no tooth loss. The final diagnosis was between Stages III and IV. After deep analysis, considering that the patient did not lose any teeth due to periodontitis and considering the current efficacy

of periodontal regeneration for infra-bony defects, the authors diagnosed the patient as Stage III (Fig. 3-left). If all the sequences recommended by this review are followed (Fig. 3-right) and it does not consider drifting/flaring for this case (not as a result of TLP, as recommended), the direct diagnosis was Stage III, similar to those found in the original article.

(b) The 2nd case was a non-smoker 34-year-old Caucasian female with obesity (BMI: 39.2 kg/m^2), taking no medication, and without any significant diseases or conditions. No tooth loss but with considerable recession in the lower anterior teeth, mainly in the left central incisor (#24 ADA / #31 FDI), which had vertical bony defect apically extended (#24 / #31). The highest PD was 7mm, and CAL was 11mm; RBL extending to the mid-third of root and beyond, with generalized mobility with localized secondary occlusal trauma (#24- #25 ADA / #31-#41 FDI). Initially, the case was qualified as Stage III or IV periodontitis; the authors defined the final diagnosis as Stage III. Observing the scenario for the classification (Fig. 4-left) and comparing it with the table suggested by this review (with modifications) (Fig. 3-right), it is possible to verify that the severity defined the case as Stage III and the complexity factors involved great part of the complexity of Stage III. Even though the complexity factors are shared between stages III and IV, summed of the secondary occlusal trauma found, the severity factor (tooth loss) was decisive in defining and keeping the case as Stage III (which was easily found compared).

2. Siqueira et al.[30] published 2 complex cases with "gray zones" for periodontitis, which were challenging to define the diagnosis. The authors provided essential thoughts for interpretation and diagnosis.

(a) The first case was an 83-year-old male with a history of congestive heart failure, atrial fibrillation, artificial aortic valve replacement, heart attack, controlled hypertension, BOP 87%, overweight (body mass index: 29.1 kg/m^2), sleep apnea, allergy to penicillin, past-smoker (quit 50 years ago). The worst CAL observed was 10mm, PD of 7mm, at #14 (ADA) or #26 (FDI). RBL was generalized, with moderate horizontal bone loss; some areas extending to the mid-third of the root; vertical bony defect was noted on #1 (ADA) or #18 (FDI), which had drifting. Four teeth were lost but for unknown reasons. Furcation class 2 (#30 ADA / #46 FDI), moderate ridge defect, >10 opposing pairs were found, with >84% of teeth affected.

Stage I	Stage II	Stage III	Stage IV
1–2 mm	3–4 mm	≥5 mm	≥5 mm
Coronal third (<15%)	Coronal third (15%–33%)	Extending to middle third of root and beyond	Extending to middle third of root and beyond
No tooth loss		≤4 teeth	≥5 teeth
• Max. probing depth ≤4 mm • Mostly horizontal bone loss	• Max. probing depth ≤5 mm • Mostly horizontal bone loss	In addition to Stage II complexity: • Probing depths ≥6 mm • Vertical bone loss ≥3 mm • Furcation involvement Class II or III • Moderate ridge defects	In addition to Stage III complexity: • Need for complex rehabilitation due to: – Masticatory dysfunction – Secondary ocelusal trauma (tooth mobility degree ≥2) – Severe ridge defects – Bite collapse, drifting, flaring – <20 remaining teeth (10 opposing pairs)

For each stage, describe extent as:
• Localized (<30% of teeth involved);
• Generalized; or
• Molar/incisor pattern

Sequence of Assessment		Periodontitis	Checklist	Stage I	Stage II	Stage III	Stage IV
1st		Interdental CAL (at site of greatest loss)	✓	1–2 mm	3–4 mm	≥5mm	≥5 mm
2nd		RBL	✓	Coronal third (<15%)	Coronal third (15%–33%)	Extending to middle third of root and	Extending to middle third of root and beyond
3rd For decision between Stage III or IV	Severity	Tooth loss or planned to be extracted (due to periodontitis) Hopeless tooth (mobility class 3)	✓	May have tooth loss		May have no tooth loss or ≤4 teeth	≥5 teeth
4th	Complexity	Local	✓	Maximum PD ≤4 mm (without recession) or PD-GM = 1 or 2 mm	Maximum PD ≤5 mm (without recession) or PD-GM = 3 or 4 mm	AnyPD	AnyPD
5th	Complexity	Local	✓	Mostly horizontal boneloss: No furcation involvement	Mostly horizontal boneloss: May have Furcation I or II; May have tooth mobility class 1	In addition to Stage II: – May have any class of Furcation involvement – May have vertical bone loss involvement – May have tooth mobility class 1 or 2 – Moderate ridge defects	In addition to Stage III: – May have any class of tooth mobility **Need for complex rehabilitation due to:** – Masticatory dysfunction – Secondary ocelusal trauma (tooth mobility degree ≥2) – Bite collapse, drifting, flaring – May have < 20 remaining teeth (10 opposing pairs) – Severe ridge defects
6th	Extent and distribution	Add to stage as descriptor	✓	For each stage, describe extent as: • Localized (< 30% of teeth involved); or • Generalized (≥ 30% of teeth involved); or • Molar/incisor pattern			

Fig. 3. Sequence followed by the authors in the article (left) and the sequence following the suggestions of this critical review (right)

Stage I	Stage II	Stage III	Stage IV
1–2 mm	3–4 mm	≥5 mm	≥5 mm
Coronal third (<15%)	Coronal third (15%–33%)	Extending to middle third of root and beyond	Extending to middle third of root and beyond
No tooth loss		≤4 teeth	≥5 teeth
• Max. probing depth ≤4 mm • Mostly horizontal bone loss	• Max. probing depth ≤5 mm • Mostly horizontal bone loss	In addition to Stage II complexity: • Probing depths ≥6 mm • Vertical bone loss ≥3 mm • Furcation involvement Class II or III • Moderate ridge defects	In addition to Stage III complexity: • Need for complex rehabilitation due to: – Masticatory dysfunction – Secondary ocelusal trauma (tooth mobility degree ≥2) – Severe ridge defects – Bite collapse, drifting, flaring – <20 remaining teeth (10 opposing pairs)

For each stage, describe extent as:
• Localized (<30% of teeth involved);
• Generalized; or
• Molar/incisor pattern

Sequence of Assessment		Periodontitis	Checklist	Stage I	Stage II	Stage III	Stage IV
1st		Interdental CAL (at site of greatest loss)	✓	1–2 mm	3–4 mm	≥5mm	≥5 mm
2nd		RBL	✓	Coronal third (<15%)	Coronal third (15%–33%)	Extending to middle third of root and	Extending to middle third of root and beyond
3rd For decision between Stage III or IV	Severity	Tooth loss or planned to be extracted (due to periodontitis) Hopeless tooth (mobility class 3)	✓	May have tooth loss		May have no tooth loss or ≤4 teeth	≥5 teeth
4th	Complexity	Local	✓	Maximum PD ≤4 mm (without recession) or PD-GM = 1 or 2 mm	Maximum PD ≤5 mm (without recession) or PD-GM = 3 or 4 mm	AnyPD	AnyPD
5th	Complexity	Local	✓	Mostly horizontal boneloss: No furcation involvement	Mostly horizontal boneloss: May have Furcation I or II; May have tooth mobility class 1	In addition to Stage II: – May have any class of Furcation involvement – May have vertical bone loss involvement – May have tooth mobility class 1 or 2 – Moderate ridge defects	In addition to Stage III: – May have any class of tooth mobility **Need for complex rehabilitation due to:** – Masticatory dysfunction – Secondary ocelusal trauma (tooth mobility degree ≥2) – Bite collapse, drifting, flaring – May have < 20 remaining teeth (10 opposing pairs) – Severe ridge defects
6th	Extent and distribution	Add to stage as descriptor	✓	For each stage, describe extent as: • Localized (< 30% of teeth involved); or • Generalized (≥ 30% of teeth involved); or • Molar/incisor pattern			

Fig. 4. Sequence followed by the authors in the article (left) and the sequence following the suggestions of this critical review (right)

Mobility degree 1 in more than 5 teeth. Traumatic occlusal forces were found (secondary occlusal trauma). The case was classified with stage III generalized periodontitis (Fig. 5-left). Therefore, observing the new table proposed and the case with a higher level of complexity, it should be classified as Stage IV. This fact is supported by the severity factors found and the cumulative complexity factors present simultaneously in stages III and IV; moreover, it is necessary to sum up two other specific complexity factors explicitly found in stage IV. All these facts justify the diagnosis of stage IV periodontitis.

(b) The 2nd case was a 73-year-old male with controlled hypertension, obesity (body mass index: 34 kg/m^2), irregular heartbeat, type 2 diabetes (HbA1c: 6.5%), and basal cell carcinoma removed years ago; partial edentulism, hyper-eruption, deep bite, severe wear, and loss of occlusal vertical dimension were found. The worst interdental CAL was 12mm (#14 ADA / #26 FDI; without adjacent tooth – not considered) and 8mm (#8 ADA / #11 FDI), with 7mm PD; RBL was generalized mild horizontal bone loss with localized severe bone loss on #5; vertical bony defects (>3mm) noted; absence of 5 teeth by unknown reason. Furcation class 2 (#15 ADA / #27 FDI), moderate ridge defect, mobility class 2. The periodontal diagnosis was stage IV generalized periodontitis (Fig. 6-left). Observing all factors reported, it is possible to easily confirm the diagnosis as Stage IV periodontitis (Fig. 6-right).

3. Steigmann et al.[31] also published 2 borderline cases in "gray zones" for periodontitis.

(a) The first case was a systemically healthy patient (66-year-old female) with a family history and diagnosis of periodontitis at the age of 14 years. The patient had signs of parafunctional bruxism and clenching, with secondary occlusal trauma, severe ridge defects, and drifting; 8 missing teeth (4 due to periodontitis). The patient had generalized interproximal CAL ≥5 mm (>30% of the teeth) with PD >6 mm; generalized RBL extending to the mid-third of the root, and three localized vertical defects (Fig. 7-left). The authors diagnosed it as stage III periodontitis, justifying there was no need for complex rehabilitation given the patient's current occlusion. Considering the new suggestions from this critical review (the presence of teeth mobilities classes 1 and 2) summed to some not well-documented points observed (description of 4 TLP in the text and it was registered 5 in the figure (Fig. 7-left); the presence of hyper-eruptions and bilateral

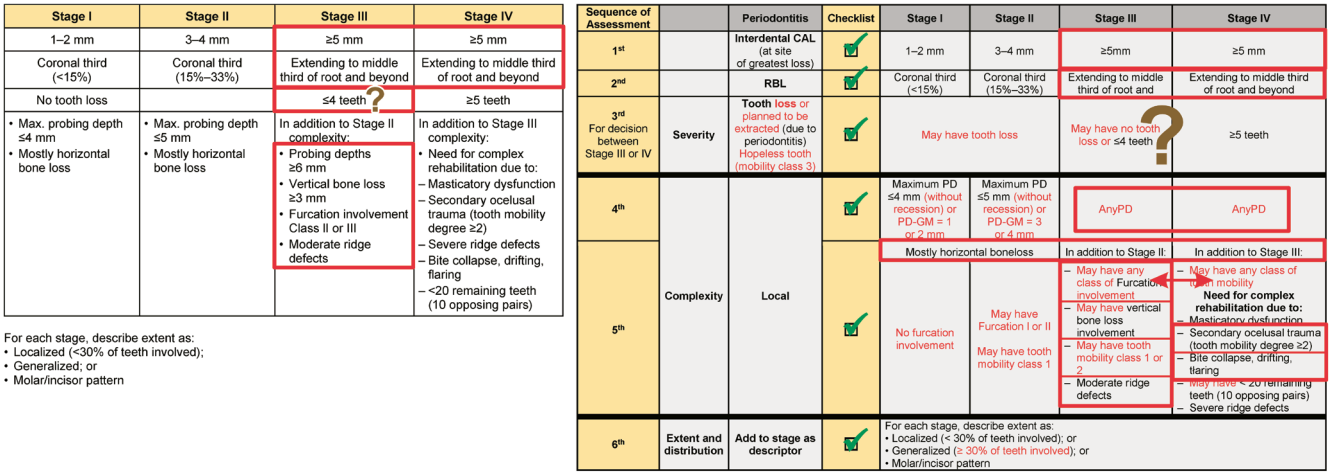

Fig. 5. Sequence followed by the authors in the article (left) and the sequence following the suggestions of this critical review (right)

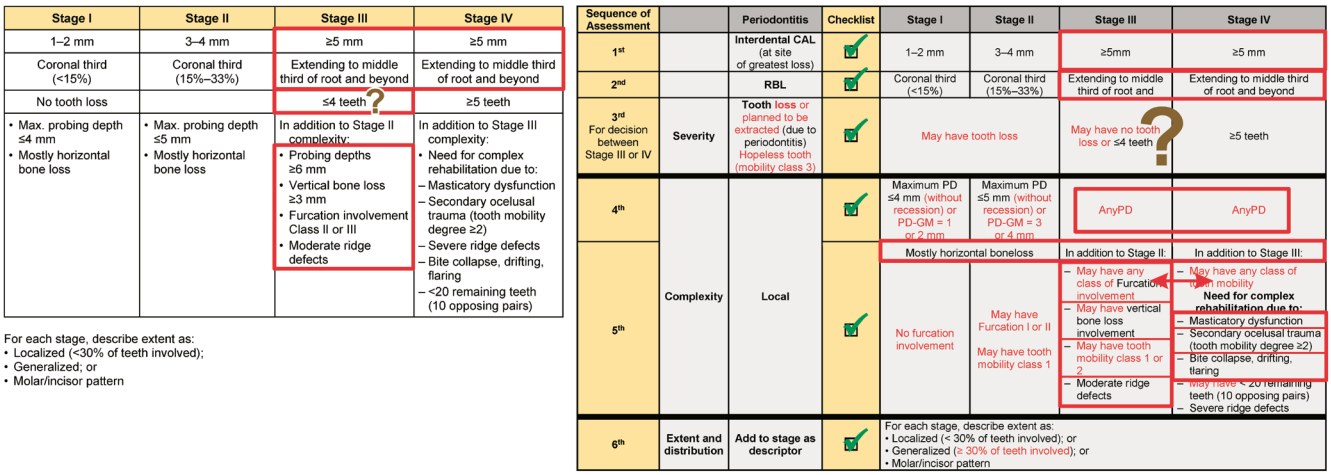

Fig. 6. Sequence followed by the authors in the article (left) and the sequence following the suggestions of this critical review (right)

altered Spee curvature), all those are factors that bring more complexity to rehabilitating the case. Then, observing the new classification and the suggestions for modification, this case fits much better in stage IV (Fig. 7-right).

(b) The 2nd case was a systemically healthy patient, a 64-year-old female with no family history of periodontitis. She had no TLP (8 missing teeth); had signs of parafunctional bruxism with secondary occlusal trauma; several periapical lesions; and one implant with peri-implant disease. The patient has generalized interproximal attachment loss ≥5 mm (>30% of the teeth), mobilities class 1 and 2, generalized horizontal bone loss with areas of vertical bony defects; and generalized horizontal RBL extending to the coronal third of the root; 8 localized vertical defects that extend to the mid-third of the root or beyond; the worst PD was 13mm and CAL of 12mm/13mm. The authors did not count hopeless teeth (6 teeth) in the initial assessment for TLP; therefore, they considered that after extractions, the patient will need complex rehabilitation (resting ten occluding pairs). The patient received the diagnosis of stage IV periodontitis (Fig. 8-left).

Observing the scenario and considering the hopeless teeth, mobility, need for complex rehabilitation, and the severity factors (favoring stage IV) and complexity factors (involving most of stage IV [it can have the complexity factors of stage III too]), the results of the new suggested table (modifications) also resulted and confirmed it as stage IV periodontitis.

Final considerations

The implementation of a new classification system typically poses challenges for its clinical application and also in education. Establishing this new classification must be seen as a process, a transitional phase, which may have adjustments for improvement to be made as effective as possible. Several articles already investigated the diagnostic accuracy of this new classification, with the presence of periodontal experts, general dentists, and students. Abrahamian et al.[27] concluded that professional clinical experience (postgraduate students, academics, and periodontal experts) is less important when applying the new classification system (no significant differences for inter- and intra-rater reliability). Likewise, Marini et al.[32] and Ravidà et al.[26] showed moderate consistency and concordance of the differently experienced examiners to the gold

Stage I	Stage II	Stage III	Stage IV
1–2 mm	3–4 mm	≥5 mm	≥5 mm
Coronal third (<15%)	Coronal third (15%–33%)	Extending to middle third of root and beyond	Extending to middle third of root and beyond
No tooth loss		≤4 teeth	≥5 teeth
• Max. probing depth ≤4 mm • Mostly horizontal bone loss	• Max. probing depth ≤5 mm • Mostly horizontal bone loss	In addition to Stage II complexity: • Probing depths ≥6 mm • Vertical bone loss ≥3 mm • Furcation involvement Class II or III • Moderate ridge defects	In addition to Stage III complexity: • Need for complex rehabilitation due to: – Masticatory dysfunction – Secondary ocelusal trauma (tooth mobility degree ≥2) – Severe ridge defects – Bite collapse, drifting, flaring – <20 remaining teeth (10 opposing pairs)

For each stage, describe extent as:
• Localized (<30% of teeth involved);
• Generalized; or
• Molar/incisor pattern

Sequence of Assessment		Periodontitis	Checklist	Stage I	Stage II	Stage III	Stage IV
1st		Interdental CAL (at site of greatest loss)	✓	1–2 mm	3–4 mm	≥5mm	≥5 mm
2nd		RBL	✓	Coronal third (<15%)	Coronal third (15%–33%)	Extending to middle third of root and	Extending to middle third of root and beyond
3rd For decision between Stage III or IV	Severity	Tooth loss or planned to be extracted (due to periodontitis) Hopeless tooth (mobility class 3)	✓	May have tooth loss		May have no tooth loss or ≤4 teeth	≥5 teeth
4th	Complexity	Local	✓	Maximum PD ≤4 mm (without recession) or PD-GM = 1 or 2 mm	Maximum PD ≤5 mm (without recession) or PD-GM = 3 or 4 mm	AnyPD	AnyPD
5th	Complexity	Local	✓	Mostly horizontal boneloss No furcation involvement	May have Furcation I or II May have tooth mobility class 1	In addition to Stage II: – May have any class of Furcation involvement – May have vertical bone loss involvement – May have tooth mobility class 1 or 2 – Moderate ridge defects	In addition to Stage III: – May have any class of tooth mobility **Need for complex rehabilitation due to:** – Masticatory dysfunction – Secondary ocelusal trauma (tooth mobility degree ≥2) – Bite collapse, drifting, flaring – May have < 20 remaining teeth (10 opposing pairs) – Severe ridge defects
6th	Extent and distribution	Add to stage as descriptor	✓	For each stage, describe extent as: • Localized (< 30% of teeth involved); or • Generalized (≥ 30% of teeth involved); or • Molar/incisor pattern			

Fig. 7. Sequence followed by the authors in the article (left) and the sequence following the suggestions of this critical review (right)

Stage I	Stage II	Stage III	Stage IV
1–2 mm	3–4 mm	≥5 mm	≥5 mm
Coronal third (<15%)	Coronal third (15%–33%)	Extending to middle third of root and beyond	Extending to middle third of root and beyond
No tooth loss		≤4 teeth	≥5 teeth
• Max. probing depth ≤4 mm • Mostly horizontal bone loss	• Max. probing depth ≤5 mm • Mostly horizontal bone loss	In addition to Stage II complexity: • Probing depths ≥6 mm • Vertical bone loss ≥3 mm • Furcation involvement Class II or III • Moderate ridge defects	In addition to Stage III complexity: • Need for complex rehabilitation due to: – Masticatory dysfunction – Secondary ocelusal trauma (tooth mobility degree ≥2) – Severe ridge defects – Bite collapse, drifting, flaring – <20 remaining teeth (10 opposing pairs)

For each stage, describe extent as:
• Localized (<30% of teeth involved);
• Generalized; or
• Molar/incisor pattern

Sequence of Assessment		Periodontitis	Checklist	Stage I	Stage II	Stage III	Stage IV
1st		Interdental CAL (at site of greatest loss)	✓	1–2 mm	3–4 mm	≥5mm	≥5 mm
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3rd For decision between Stage III or IV	Severity	Tooth loss or planned to be extracted (due to periodontitis) Hopeless tooth (mobility class 3)	✓	May have tooth loss		May have no tooth loss or ≤4 teeth	≥5 teeth
4th	Complexity	Local	✓	Maximum PD ≤4 mm (without recession) or PD-GM = 1 or 2 mm	Maximum PD ≤5 mm (without recession) or PD-GM = 3 or 4 mm	AnyPD	AnyPD
5th	Complexity	Local	✓	Mostly horizontal boneloss No furcation involvement	May have Furcation I or II May have tooth mobility class 1	In addition to Stage II: – May have any class of Furcation involvement – May have vertical bone loss involvement – May have tooth mobility class 1 or 2 – Moderate ridge defects	In addition to Stage III: – May have any class of tooth mobility **Need for complex rehabilitation due to:** – Masticatory dysfunction – Secondary ocelusal trauma (tooth mobility degree ≥2) – Bite collapse, drifting, flaring – May have < 20 remaining teeth (10 opposing pairs) – Severe ridge defects
6th	Extent and distribution	Add to stage as descriptor	✓	For each stage, describe extent as: • Localized (< 30% of teeth involved); or • Generalized (≥ 30% of teeth involved); or • Molar/incisor pattern			

Fig. 8. Sequence followed by the authors in the article (left) and the sequence following the suggestions of this critical review (right)

standard. Therefore, it is recommended that new investigations apply this new flowchart/suggested modifications in order to validate the decision-making periodontal diagnosis, which intends to facilitate the periodontal clinical assessment, even if it seems complex at the beginning.

Once again, our suggestion in this critical review is to organize the knowledge better and keep the same sequence/assessment parameters for all stages of periodontitis. Then, it is strongly recommended to check and keep the parameters analyzed cumulatively: first severity and after complexity, following the sequence: 1st – CAL (also obtain PD and GM), 2nd – RBL, 3rd – TLP (for decision between Stages III and IV); then, the complexity factors, as demonstrated in Fig. 2. It is important to highlight that if the patient has TLP (3 teeth), but the worst site CAL is 4mm, the Stage must be kept on Stage II, respecting the cumulative sequence suggested (CAL is more important for the case scenario than TLP).

It is worth remembering this review suggests that never one complexity parameter can overcome a severity parameter to change the Stage obtained through CAL (1st), RBL (2nd), and TLP (3rd). An exception must be respected only for complexity factors between Stages III and IV that can change the initial Stage III or IV obtained by the severity analysis, but only between themselves, according to the complexity found in the case (Fig. 2).

Then, after reading all the articles and observing the flowcharts and sequence proposed, in order to improve the clinician's decision-making diagnosis, this critical review developed and included within this article a new complete periodontal flowchart (based on the included articles), suggesting a full sequence for periodontal assessment, already including the modifications proposed on Staging (Periodontitis) (Fig. 9).

Unquestionably, the new Classification of Periodontal and Peri-Implant Diseases and Conditions (2018) is one of the most interesting evolutions of classification systems that permit the diagnosis of periodontal/peri-implant diseases. Therefore, observing the difficulty around the world in staging periodontitis, this critical review deeply analyzed this question.

This critical review suggests, specifically, that complexity parameters cannot overcome the severity parameters and to strictly follow the sequence for diagnosing: CAL (1st), RBL (2nd), and TLP (3rd), where the 1st cannot be surpassed by the 2nd or 3rd, and similarly, the 2nd cannot be surpassed by the 3rd parameter. An exception is permitted only for complexity factors between Stages III and IV that can change the initial Stage (III or IV) obtained through the severity analysis, but only between themselves (Stages III and IV), according to the complexity found. Moreover, for patients without tooth loss or with TLP of ≤4 teeth (without need for complex rehabilitation) and presenting any drifting or flaring or a secondary traumatic occlusion, it cannot be a justification for moving the diagnosis from Stage III to Stage IV.

Furthermore, some modifications for staging periodontitis are also suggested:

– for severity:
  (1) TLP summed up a hopeless tooth to be extracted (bone present only in the apical third of the root and mobility class 3): Stages I and II may have tooth loss;
– for complexity:
  (1) Stage I: should be considered PD ≤4 mm without recession or the calculation PD minus GM resulting in 1 or 2mm; and this stage cannot have furcation involvement;
  (2) Stage II: should be considered PD ≤5 mm without recession or the calculation PD minus GM resulting in 3 or 4mm; and may have furcation I or II involvement and mobility class 1;
  (3) Stage III: this stage can have any PD; may have any class of furcation involvement; may have vertical bone loss; and may have tooth mobility 1 or 2;
  (4) Stage IV: this stage can have any PD; may have any class of tooth mobility; and may have < 20 remaining teeth.

Conclusions

It was possible to conclude that there is instability and "gray zones" in the staging step of Periodontitis. This is due to a lack of priority and an organized order sequence, where the most important parameters were overcome by others. Thus, this critical review intends to create and stimulate a debate for improving specific points of the new classification, specifically in staging periodontitis. Then, we introduced a new table with the modifications suggested and a new full flowchart for the sequence of the periodontal diagnosis. However, it is required that experts in periodontics critically assess and validate the modifications proposed to verify how they clinically facilitate finding the periodontal diagnosis.

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.

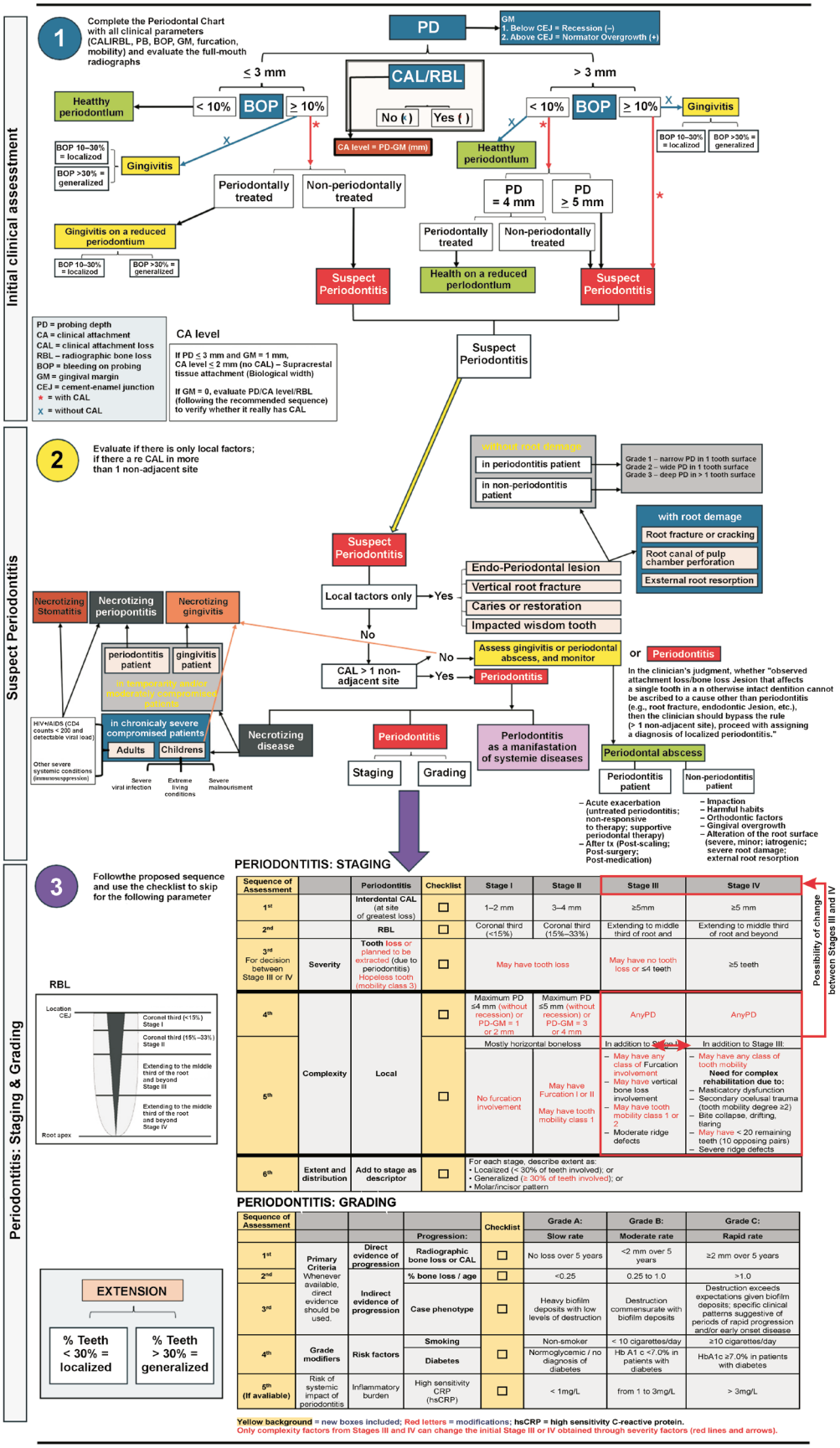

Fig. 9. New flowchart for periodontal diagnosis following the articles included in this review with suggested adaptations and modifications

ORCID iDs

Gustavo Vicentis de Oliveira Fernandes
https://orcid.org/0000-0003-3022-4390
Juliana Campos Hasse Fernandes
https://orcid.org/0000-0001-7603-3544

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Research letter

Assessment of the relationship between sleep bruxism, reported pain and headache, selected health factors, and general health conditions among temporomandibular disorder patients: A preliminary report

Sylwia Orzeszek[1,A–D], Helena Martynowicz[2,A,B,E,F], Joanna Smardz[1,A,E], Katarzyna Kresse-Walczak[3,E,F], Anna Wojakowska[2,B,C], Wojciech Bombała[4,B,C], Marta Bort[1,D], Mieszko Wieckiewicz[1,A–C,E,F]

[1] Outpatient Clinic for Temporomandibular Disorders, Department of Experimental Dentistry, Wroclaw Medical University, Poland
[2] Clinical Department of Diabetology, Hypertension and Internal Diseases, Institute of Internal Diseases, Wroclaw Medical University, Poland
[3] Department of Prosthodontics, Carl Gustav Carus Faculty of Medicine, Dresden University of Technology, Germany
[4] Statistical Analysis Center, Wroclaw Medical University, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2025;62(2):393–399

Address for correspondence
Sylwia Orzeszek
E-mail: sylwia.winiewska87@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on July 17, 2024
Reviewed on July 30, 2024
Accepted on August 31, 2024

Published online on April 30, 2025

Cite as
Orzeszek S, Martynowicz H, Smardz J, et al. Assessment of the relationship between sleep bruxism, reported pain and headache, selected health factors, and general health conditions among temporomandibular disorder patients: A preliminary report. *Dent Med Probl.* 2025;62(2):393–399. doi:10.17219/dmp/192824

DOI
10.17219/dmp/192824

Abstract

Background. Temporomandibular disorders (TMD) constitute a serious health problem that can have a negative effect on patients' lives, impair work performance, and result in work absences and restrictions in daily activities. Therefore, it is of great importance not only to employ appropriate diagnostic and therapeutic procedures in the case of patients suffering from TMD and/or sleep bruxism (SB), but also to analyze the impact of different medical and non-medical factors on the occurrence of such conditions, as the proper modification of these factors may mitigate the severity of patients' symptoms.

Objectives. The present preliminary study aimed to assess the relationship between SB, reported pain and headache, selected health factors, and general health conditions among TMD patients.

Material and methods. A total of 114 patients from the Outpatient Clinic for Temporomandibular Disorders in Wroclaw, Poland, had single-night video-polysomnography (PSG) performed. The patients completed questionnaires about their pain experience, headache and health condition, including the presence or history of diseases such as hypothyroidism, arterial hypertension, cancer, diabetes, myocardial infarction, stroke, gastroesophageal reflux disease (GERD), and were asked about the frequency of alcohol consumption, smoking, caffeine use, and physical activity. The collected data was statistically analyzed.

Results. It seems that among patients with TMD, a history of cancer and GERD may have an impact on the experience of pain and headache. Smoking was significantly associated with the occurrence and intensity of SB.

Conclusions. A few habits, health factors and general health conditions of patients with TMD are associated with SB, and reported pain and headache, but this relationship requires further research conducted on a larger study group.

Keywords: habits, TMD, general health, headache, sleep bruxism

Highlights

- A few habits, health factors, and general health conditions of patients with temporomandibular disorders (TMD) are associated with sleep bruxism (SB), and reported pain and headache.
- A history of cancer and gastroesophageal reflux disease (GERD) may have an impact on the experience of pain and headache.
- Sleep bruxism is significantly associated with smoking.
- The investigated relationships require further research conducted on a larger study group.

Introduction

Temporomandibular disorders (TMD) include masticatory muscle or temporomandibular joint (TMJ) pain, limited jaw movements, joint sounds, such as clicking or crepitus, myofascial pain, and headache.[1] They are considered the 2nd most common cause of oral and facial region pain, following odontalgic pain.[2] Additionally, it is ranked as the 2nd main cause of musculoskeletal pain, after chronic low-back pain.[1] The prevalence of TMD is estimated to be between 5% and 12% in the general population, with a higher incidence of up to 30% among young adults.[3] In the Polish population, approx. 55.9% of individuals experience at least one symptom of TMD.[4] Gender differences are apparent, with women more commonly affected by TMD in the Polish population.[4]

Sleep bruxism (SB) is a repetitive jaw muscle activity characterized by the clenching and/or grinding of the teeth, which is classified under sleep-related movement disorders (SRMD) in the International Classification of Sleep Disorders – Third Edition (ICSD-3).[5] Sleep bruxism is a common phenomenon worldwide, with a prevalence of 8–13% in the general population.[6]

Orofacial pain (OFP) encompasses a heterogeneous group of conditions, such as dental, mucosal, musculoskeletal, neurovascular, and neuropathic pain.[7] In OFP and headache, chronic pain is defined as the pain occurring more than 15 days per month and lasting more than 4 h per day for at least the last 3 months.[8,9]

The aim of this preliminary study was to assess the relationship between SB, reported pain and headache, selected health factors, and general health conditions among TMD patients.

Material and methods

Participants

The project was approved by the Ethics Committee of Wroclaw Medical University, Poland (KB-794/2019). All of the study participants were fully informed about the purpose of the study and agreed to take part in it. The study was carried out following the Declaration of Helsinki for experiments involving humans.

Patients with TMD were examined using single-night video-polysomnography (PSG). They were also interviewed about their health condition, with regard to the presence or history of diseases such as hypothyroidism, arterial hypertension, cancer, diabetes, myocardial infarction, stroke, gastroesophageal reflux disease (GERD), and were asked about the frequency of alcohol consumption, smoking, caffeine use, and physical activity.

Inclusion criteria

The patients were included in the study if they met the following criteria: age above 18 years; the presence of OFP or headache, as determined using the Graded Chronic Pain Scale (GCPS), the Headache Impact Test-6 (HIT-6), the Migraine Disability Assessment (MIDAS), the short-form McGill Pain Questionnaire (SF-MPQ), and the TMD pain screener; and willingness to participate in the study.

Exclusion criteria

The exclusion criteria were as follows: addiction to a drug or a medication; using medicines that significantly affect the function of the nervous and muscular systems; severe systemic diseases and severe mental disorders, including significant mental disabilities or the presence of cancer; less than 4 h of sleep recorded using PSG; pregnancy; the presence and treatment of sleep apnea; and the lack of consent to participate in the study.

Video-polysomnography examination

The intensity of bruxism was assessed using the bruxism episode index (BEI), indicating the number of bruxism episodes per hour of sleep. The cut-off points were determined in accordance with the guidelines of the American Academy of Sleep Medicine (AASM) ICSD-3: BEI < 2 – irrelevant SB; BEI of 2–4 – mild to moderate SB; and BEI > 4 – severe SB.[10]

Pain assessment

Pain intensity was determined using validated questionnaires, such as SF-MPQ,[11] GCPS,[12] MIDAS,[13] HIT-6,[14] and the TMD pain screener.[15]

Health questionnaire

The patients from the study group were asked to complete a questionnaire on general health and habits, which consisted of 10 parts regarding the patient's medical history, diseases in the family, the medications taken, the patient's eating habits, alcohol consumption, smoking, caffeine use, and physical activity, as well as demographic data.

Database

The participants' medical history, questionnaire responses and PSG data were entered into a database using Microsoft Excel (Microsoft Corporation, Redmond, USA). The elements of the database were subjected to statistical analysis.

Statistical analysis

The *U* Mann–Whitney correlation coefficient was used to check the relationships between the variables. The choice of the coefficient was dictated by the fact that the variables did not have a normal distribution. A *p*-value for a correlation coefficient below 0.05 was considered significant. Statistica™, v.13.1 (StatSoft, Krakow, Poland), was used for the statistical analysis of the data. The prediction of the sample size (*N*) was established with the use of the power.cor function and the genefu package (https://rdrr.io/bioc/genefu/man/power.cor.html).

Results

Characteristics of the study sample

Age and gender

A total of 114 adult participants were included in the study (72 women and 42 men). The female-to-male ratio in the group was 1.71:1. All participants were Caucasians, aged 21–71 years (mean age: 37.67 years).

Reported pain

The level of pain severity was determined by the participants using 4 independent questionnaires, as illustrated in Table 1.

Severity of sleep bruxism

The percentage distribution of the BEI values according to gender is shown in Table 1.

Reported selected health factors and general health conditions

Hypothyroidism occurred in 2 study participants (1.75%). Arterial hypertension was reported by 5 patients (4.39%). In all cases, the patients declared that they took medications regularly and their blood pressure was regulated. Four patients (3.51%) had an oncological history. Three respondents were treated for diabetes (2.63%). The study group did not include any patients reporting a history of myocardial infarction or stroke. However, as many as 33 patients (28.95%) reported the occurrence of GERD.

Forty-two patients admitted to drinking alcohol, which constituted 36.84% of the study group; 5 patients (4.39%)

Table 1. Levels of pain severity and the bruxism episode index (BEI) values according to gender

Variable		Examined women	Examined men
GCPS	grade 0	8.54	33.34
	grade 1	56.95	54.76
	grade 2a, 2b	19.44	7.14
	grade 3	12.50	4.76
	grade 4	2.57	0.00
HIT-6	no or little impact	43.11	64.29
	slight impact	8.33	16.67
	significant impact	5.56	11.90
	severe impact	43.00	7.14
MIDAS	grade I	42.86	63.63
	grade II	19.05	9.10
	grade III	14.29	18.17
	grade IV	23.80	9.10
SF-MPQ	total value ≤5	59.72	64.91
	total value >5	40.28	35.09
TMD pain screener	total value ≤3	34.43	53.41
	total value >3	65.57	46.59
BEI	>2	25.00	13.89
	2–4	31.25	13.89
	>4	43.75	72.22

Data presented as percentage (%).
GCPS – Graded Chronic Pain Scale; HIT-6 – Headache Impact Test-6; MIDAS – Migraine Disability Assessment; SF-MPQ – short-form McGill Pain Questionnaire; TMD pain screener – temporomandibular disorder pain screener.

stated that they consumed alcohol regularly and 37 patients (32.46%) only occasionally. There were 12 cigarette smokers, representing 10.53% of the study group. Twenty patients (17.54%) admitted to drinking coffee regularly, while 6 patients (5.26%) drank coffee occasionally, which gives a total of 26 patients, i.e., 22.8% of the respondents. Physical activity was reported by 64 patients (56.14%), with only 11 patients (9.65%) exercising every day, 19 patients (16.67%) reporting physical activity 3–4 times a week, 28 patients (24.56%) practicing 1–2 times a week, and 6 patients (5.26%) practicing less than once a week.

Reported selected health factors and general health conditions and reported pain

The data on the environmental factors were compared with the level of pain reported by the patients in the GCPS, MIDAS, HIT-6, SF-MPQ questionnaires, and the TMD pain screener, using the Mann–Whitney *U* test (Table 2).

There were no statistically significant relationships between the level of pain and hypothyroidism, arterial hypertension, diabetes, alcohol consumption, smoking, caffeine use, and physical activity ($p > 0.05$).

However, the Mann–Whitney *U* test showed a statistically significant relationship between reported pain and a history of cancer. This relationship occurred for the HIT-6 questionnaire ($p = 0.037$) and the MIDAS questionnaire ($p = 0.032$). No statistically significant relationships for the GCPS and SF-MPQ questionnaires, and the TMD pain screener were noted ($p > 0.05$).

We also noticed a statistically significant relationship between the level of pain reported by the patients and the co-occurring GERD. The last correlation concerns the GCPS questionnaire ($p = 0.039$). This means that patients suffering from GERD report higher levels of pain and pain-related disability. There were no such statistically significant relationships with regard to the HIT-6, MIDAS and SF-MPQ questionnaires, and the TMD pain screener ($p > 0.05$).

Reported selected health factors and general health conditions and sleep bruxism

Statistical analysis with the use of the Mann–Whitney *U* test was also performed to investigate the relationships between hypothyroidism, arterial hypertension, cancer, diabetes, GERD, alcohol consumption, smoking, caffeine use, and physical activity and the occurrence and severity of SB (Table 2). The analysis showed that only in the case of smoking can we speak of a statistically significant relationship ($p = 0.016$).

Among cigarette smokers, only 1 patient had BEI < 2 (8.33% of all smokers). Similarly, mild to moderate SB (BEI of 2–4) occurred in 1 patient (8.33%), while 10 patients (83.33%) were classified as presenting severe bruxism due to BEI > 4.

Discussion

Østensjø et al. found that the factors influencing the occurrence of painful TMD are female gender, living in urban areas, complaining of severe menstrual pain, and frequent headaches.[16] Women not only reported pain more often, but the intensity of the pain was greater.[16] The results regarding gender are consistent with the findings of our study, in which the predominant group were women, and in this group, the pain was more intense and caused greater disability than in men. However, the authors of the abovementioned study reported that physical activity could have an alleviating effect on the pain felt.[16] Many other studies also point to the reduction of pain in the group of people regularly engaging in physical activity.[17] In our study, the relationship between physical activity and reported pain was not statistically significant, although physical activity certainly had a greater impact on pain reported in GCPS ($p = 0.065$) than on the occurrence of SB ($p = 0.271$).

A large study started in 2006 – the OPPERA project (Orofacial Pain: Prospective Evaluation and Risk Assessment) – aimed at identifying risk factors for the development of painful TMD.[18] According to this research, TMD develop at a disproportionate rate in people with relatively poor health, whether in the form of comorbidities or otherwise pain, poor sleep quality or smoking.[18]

Grozdinska et al. in their study conducted on a group of 119 women, including 52 women in the study group diagnosed with Hashimoto's disease and 67 healthy people in the control group, noticed markedly increased incidence of TMD in the study group.[19] Muscle pain and stiffness were observed in 45 patients from the study group (86.5%) and 33 (63.5%) had disk displacement with repositioning.[19] The results of that study showed that the prevalence of TMD, especially of muscle disorders in patients with Hashimoto's thyroiditis, is higher than in the control group ($p < 0.001$).[19] Our research did not show statistically significant relationships between reported pain and thyroid diseases, or between SB and thyroid diseases, but the study group included only 2 patients with hypothyroidism, so it was impossible to draw clear and valuable conclusions regarding this dependence.

Miettinen et al. in their work, in which the study group consisted of 8,678 participants (148 women and 8,530 men), tested with the use of questionnaires TMD symptoms, health behavior and background/demographic factors, and noticed higher prevalence for all TMD symptoms (except TMJ clicking) in the female population.[20] Smoking was significantly associated with TMD symptoms, except for TMJ clicking. The consumption of alcohol at least once a week was significantly associated with facial pain, TMJ pain and TMJ clicking. The use of snuff was significantly associated with facial pain.[20] The results presented by Miettinen et al. are consistent with those of Sanders et al., who showed that smoking

Table 2. Summary of the statistical relationships between the intensity of pain and bruxism and the general health of patients, as well as the factors influencing their health

Variables	Sum of ranks group I	Sum of ranks group II	*U*	*Z*	*p*-value	*Z* corrected	*p*-value
GCPS & hypothyroidism	3,252.500	752.500	551.500	−0.34190	0.732	−0.34337	0.731
HIT-6 & hypothyroidism	2,999.000	829.000	443.000	−1.36405	0.173	−1.36629	0.172
MIDAS & hypothyroidism	2,614.000	707.000	403.000	−1.11247	0.266	−1.13166	0.258
SF-MPQ & hypothyroidism	3,253.500	841.500	478.500	−1.19254	0.233	−1.21936	0.223
TMD pain screener & hypothyroidism	2,965.000	690.000	480.000	−0.51299	0.608	−0.51867	0.604
GCPS & arterial hypertension	3,434.500	570.500	431.500	−0.36037	0.719	−0.36192	0.717
HIT-6 & arterial hypertension	3,344.500	483.500	417.500	0.00000	1.000	0.00000	1.000
MIDAS & arterial hypertension	2,907.000	414.000	348.000	0.50319	0.615	0.51187	0.609
SF-MPQ & arterial hypertension	3,389.000	706.000	308.000	−1.89318	0.058	−1.93576	0.053
TMD pain screener & arterial hypertension	3,106.500	548.500	405.500	−0.40387	0.686	−0.40834	0.683
GCPS & cancer	3,758.500	246.500	103.500	−1.30699	0.191	−1.31260	0.189
HIT-6 & cancer	3,606.000	222.000	36.000	−2.08196	0.037	−2.08538	0.037*
MIDAS & cancer	3,113.000	208.000	32.000	−2.11316	0.035	−2.14961	0.032*
SF-MPQ & cancer	3,837.000	258.000	96.000	−1.47822	0.139	−1.51146	0.131
TMD pain screener & cancer	3,453.000	202.000	132.000	−0.61220	0.540	−0.61898	0.536
GCPS & diabetes	3,573.000	82.000	76.000	1.10746	0.268	1.11199	0.266
HIT-6 & diabetes	3,333.000	153.000	93.000	−0.64653	0.518	−0.64754	0.517
MIDAS & diabetes	3,033.500	126.500	107.500	−0.15390	0.878	−0.15676	0.875
SF-MPQ & diabetes	3,601.000	140.000	115.000	−0.21182	0.832	−0.21635	0.829
TMD pain screener & diabetes	3,418.000	68.000	62.000	1.40286	0.161	1.41895	0.156
GCPS & GERD	2,392.500	1,177.500	616.500	2.05615	0.040	2.06392	0.039*
HIT-6 & GERD	2,074.000	1,329.000	696.000	−0.80385	0.421	−0.80509	0.421
MIDAS & GERD	1,923.500	1,236.500	648.500	−0.77299	0.440	−0.78738	0.431
SF-MPQ & GERD	2,354.000	1,301.000	740.000	1.05955	0.289	1.08076	0.280
TMD pain screener & GERD	2,135.000	1,268.000	740.000	0.56560	0.572	0.57190	0.567
GCPS & alcohol consumption	2,920.500	907.500	574.500	−0.72942	0.466	−0.73271	0.464
HIT-6 & alcohol consumption	2,731.500	923.500	453.500	−1.60271	0.109	−1.60509	0.108
MIDAS & alcohol consumption	2,494.000	827.000	414.000	−1.50189	0.133	−1.52780	0.127
SF-MPQ & alcohol consumption	2,973.500	942.500	627.500	−0.51776	0.605	−0.52806	0.597
TMD pain screener & alcohol consumption	2,787.500	867.500	576.500	−0.52743	0.598	−0.53327	0.594
GCPS & smoking	3,401.000	427.000	349.000	1.23707	0.216	1.24265	0.214
HIT-6 & smoking	3,235.500	419.500	341.500	1.21160	0.226	1.21341	0.225
MIDAS & smoking	3,013.500	307.500	252.500	1.46438	0.143	1.48964	0.136
SF-MPQ & smoking	3,516.000	400.000	322.000	1.62323	0.105	1.65550	0.098
TMD pain screener & smoking	3,268.000	387.000	321.000	1.11943	0.263	1.13183	0.258
GCPS & caffeine use	3,102.000	726.000	516.000	1.54848	0.122	1.55546	0.120
HIT-6 & caffeine use	2,785.000	870.000	507.000	−1.02724	0.304	−1.02877	0.304
MIDAS & caffeine use	2,431.500	889.500	478.500	−1.22604	0.220	−1.24719	0.212
SF-MPQ & caffeine use	3,130.500	785.500	575.500	1.03552	0.300	1.05611	0.291
TMD pain screener & caffeine use	2,828.500	826.500	617.500	−0.09494	0.924	−0.09660	0.924
GCPS & physical activity	1,203.500	2,624.500	544.500	1.83835	0.066	1.84578	0.065
HIT-6 & physical activity	1,105.000	2,550.000	597.000	1.14252	0.253	1.14422	0.253
MIDAS & physical activity	855.000	2,385.000	555.000	0.49444	0.621	0.50330	0.615
SF-MPQ & physical activity	1,186.000	2,730.000	585.000	1.53847	0.124	1.57219	0.116
TMD pain screener & physical activity	833.500	2,736.500	591.500	0.27264	0.785	0.27571	0.783
BEI & hypothyroidism	2,936.500	633.5000	513.500	0.04088	0.967	0.04088	0.967
BEI & arterial hypertension	3,141.500	428.500	366.500	−0.04144	0.967	−0.04144	0.967
BEI & cancer	3,436.000	134.000	124.000	0.74565	0.456	0.74576	0.456
BEI & diabetes	–	–	0.000	0.00000	1.000	0.00000	1.000
BEI & GERD	1,818.500	1,341.500	690.500	−0.60919	0.542	−0.60927	0.542
BEI & alcohol consumption	2,731.500	671.500	518.500	0.38319	0.702	0.38325	0.702
BEI & smoking	2,769.500	633.500	213.500	−2.40143	0.016	−2.40181	0.016*
BEI & caffeine use	2,654.000	749.000	559.000	0.42862	0.668	0.42868	0.668
BEI & physical activity	767.500	2,635.500	536.500	−1.09956	0.272	−1.09971	0.271

GERD – gastroesophageal reflux disease; * statistically significant (Mann–Whitney *U* test with the continuity correction).

was associated with TMD in women, but only in young adulthood.[21] We can find contradictory results in Wänman's study, which concluded that smoking was not related to the presence or development of the signs and symptoms of TMD among an adult population (30–65 years of age).[22] In their research, Castroflorio et al. show that smoking, more than alcohol, seems to have an impact on the occurrence of SB.[23] Similar conclusions come from our study. The Mann–Whitney *U* test confirms a relationship between smoking and SB at $p = 0.016$. However, with regard to pain and smoking, no statistically significant relationship was found ($p > 0.05$). Alcohol consumed both occasionally and regularly did not increase the intensity of pain ($p > 0.05$) and does not seem to have a significant impact on the occurrence of SB ($p > 0.05$). Also, the amount of coffee consumed did not have a significant influence on the intensity of pain, or an increase in the frequency and intensity of SB ($p > 0.05$), which is contrary to the findings of Frosztega et al., who reported that habitual coffee consumption was a risk factor for an increased intensity of SB.[24]

Our research shows that GERD may be associated with pain in patients with TMD ($p = 0.039$). Similar conclusions come from the work of Li et al., who report that symptomatic GERD is associated with chronic TMD pain.[25] According to previously conducted research, the acidification of the esophagus not only increases the rhythm activity of the masticatory muscles, as well as the clenching and/or grinding of the teeth during sleep,[26] but also increases the activity of the muscles during waking hours.[27] Also, Nota et al. in their systematic review indicate a significant association between GERD and bruxism, mostly awake bruxism (AB).[28]

Kanclerska et al. emphasize the role of the dentist in making a proper diagnosis and providing care to the patient.[29] They state that dental screening is necessary for patients with arterial hypertension, especially those presenting with the symptoms of SB. According to the researchers, nonapneic hypertensives showed greater SB intensity, altered sleep architecture, increased snoring, and decreased mean oxygen saturation as compared to normotensives.[29] Martynowicz et al. in their study aimed to assess the intensity of SB in patients with arterial hypertension.[30] A total of 70 adults participated in this study: 35 patients with hypertension (the study group); and 35 normotensive subjects (the control group). Data was recorded using a portable home cardiorespiratory polygraphy device. The BEI in the study group was found to be significantly higher as compared to the control group.[30,31] In our study, we did not demonstrate statistically significant relationships between arterial hypertension and reported pain, or between arterial hypertension and SB. However, it is worth mentioning at this point that the study group included an insufficient number of patients suffering from arterial hypertension to correctly determine this relationship, especially in the absence of a control group.

The study conducted by our research team has few limitations, but it allows us to outline the direction of further work on the topic discussed. First of all, a limitation of the study are small groups in terms of the incidence of hypothyroidism, arterial hypertension, cancer, diabetes, and GERD among patients with TMD or SB, and patients with a history of myocardial infarction or stroke. An additional limitation is the lack of a control group. Another limitation of the study is the fact that the occurrence and severity of pain were determined using questionnaires, as was TMD determined using the TMD pain screener. The occurrence of bruxism and the analysis of sleep quality were determined through a PSG examination, which is a great advantage of this study, while the PSG recording was carried out on the first night spent in hospital, without an adaptation night. Therefore, we are aware that the study has a high risk of interpretation bias and is a preliminary report.

Conclusions

A few habits, health factors and general health conditions of patients with TMD are associated with SB, and reported pain and headache, but this relationship requires further research conducted on a larger study group.

Ethics approval and consent to participate

The project was approved by the Ethics Committee of Wroclaw Medical University, Poland (KB-794/2019). All of the study participants were fully informed about the purpose of the study and agreed to take part in it.

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.

ORCID iDs

Sylwia Orzeszek https://orcid.org/0000-0002-3139-5696
Helena Martynowicz https://orcid.org/0000-0003-1283-8460
Joanna Smardz https://orcid.org/0000-0003-0004-3134
Katarzyna Kresse-Walczak https://orcid.org/0000-0002-0935-1785
Anna Wojakowska https://orcid.org/0000-0002-4541-1239
Wojciech Bombała https://orcid.org/0009-0001-9989-8337
Marta Bort https://orcid.org/0009-0002-7032-0379
Mieszko Wieckiewicz https://orcid.org/0000-0003-4953-7143

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