Dental
and Medical Problems

Wroclaw
MEDICAL UNIVERSITY

Dental
and Medical Problems

Manuscript editing

Joanna Gudarowska
Jolanta Prazeres

Editorial Policy

Ministerstwo
Edukacji i Nauki

Contents

Research letter

Brittle dental ceramics: A challenge in dentistry

João Paulo Mendes Tribst[1,A,D–F], Luiz Felipe Valandro[2,A,D–F], Amanda Maria de Oliveira Dal Piva[3,A,D–F]

[1] Department of Oral Regenerative Medicine, Academic Centre for Dentistry Amsterdam (ACTA), the Netherlands
[2] Post-Graduate Program in Oral Sciences, Federal University of Santa Maria (UFSM), Brazil
[3] Department of Dental Materials, Academic Centre for Dentistry Amsterdam (ACTA), the Netherlands

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

Keywords: fatigue, ceramics, editorial

The challenge of dental ceramic degradation necessitates innovative technology, rigorous testing and proactive dental care, demanding collaboration between researchers, dentists and patients to ensure durable and reliable dental restorations.

Dental ceramics are a groundbreaking advancement in the field of dentistry, offering not only functional dental restorations but also aesthetically pleasing solutions to patients.[1,2] However, the fatigue of dental ceramics remains a persistent challenge for dental practitioners and patients.[2,3] This phenomenon, characterized by the gradual degradation of the ceramic's properties over time, demands urgent attention from the dental community. Aging can also affect other biomaterials, such as dental composites and cements.[4,5] However, in brittle materials like ceramics, its effect can result in a reduction of the material's properties by over 50% of the original value.[6]

Dental ceramics are often considered an ideal choice for dental restorations due to their natural appearance, biocompatibility and durability.[7] They mimic the properties of natural teeth, making them an attractive option for patients seeking long-lasting dental solutions. However, the reality is far more complex. Dental ceramics are subjected to a multitude of challenging conditions within the oral environment. These include fluctuating temperatures, acidic pH levels and intense masticatory forces.[8] These factors contribute to the deterioration of ceramic restorations over time, resulting in cracks, chipping, and, in severe cases, bulk failure.[9,10]

Deterioration of dental ceramics compromises the structural integrity of the restoration, jeopardizing patients' oral health and placing a financial burden on both patients and the healthcare system. Addressing this issue requires a multifaceted approach that combines innovative research, advanced material engineering and enhanced clinical practices to repair and improve aged restorations.[11]

Dentists and researchers must collaborate to develop ceramics with improved mechanical properties and enhanced resistance to crack propagation. Incorporating nanoparticles, adjusting material composition and implementing new designs are promising innovations in nanotechnology for creating dental ceramics that can withstand the challenges of the oral environment for extended

periods.[12] Additionally, rigorous testing methodologies must be established to assess the long-term performance and durability of these materials before introducing them into clinical practice. Fatigue cycling, including accelerated life tests and the measurement of stress-life curves (S-N), are fundamental concepts in the field of biomaterials that should be encouraged to predict the fatigue life of dental ceramics.[2] In vitro tests that apply intermittent cyclic loading are essential to induce mechanical fatigue of structures. These tests should be performed to predict mechanical behavior, particularly when interactions occur between recurrent stresses from fatigue regimens and critical defects observed at the inner and outer surfaces of ceramic restorations. The literature has shown that critical defects introduced during finishing/polishing of outer surfaces or grinding of the inner surfaces significantly affect the mechanical behavior of ceramic restorations.[2–7] For instance, grinding the inner surface of ceramic restorations can markedly reduce their mechanical fatigue behavior.[7,12] Moreover, inner surface treatments are the primary factor influencing the mechanical behavior. Promoting maximum adhesion between the materials/substrates is a must to induce a better mechanical behavior.[13] Therefore, dentists are strongly encouraged to prevent the introduction of defects and/or to manage them through proper finishing, polishing and surface treatment of the ceramic surfaces. This will mitigate their deleterious effects and optimize adhesion and its positive effects.[4]

Fractography analysis and finite element analysis (FEA) are indispensable tools for the comprehensive study of the fatigue behavior of dental ceramics. They provide valuable insights that can significantly improve the validity and reliability of fatigue results (Fig. 1). The meticulous examination of fracture surfaces enables to identify specific fracture features that can indicate the correct direction of crack propagation.[13] These patterns can be used by researchers to determine the vulnerabilities of ceramics under cyclic loading, which can guide the improvements in design and composition. Moreover, identifying the primary causes of failures, such as material defects, manufacturing processes, or bonding techniques, allows for addressing these issues at their source, strengthening the durability of dental restorations.[14]

Dental professionals play a pivotal role in reducing the effects of ceramic fatigue. Education and training programs should be intensified to ensure that practitioners are well-versed in the latest advancements in dental ceramics. Computer-aided design and computer-aided manufacturing (CAD/CAM) techniques can be used to create precise ceramic restorations, which can reduce the likelihood of premature failures through topology optimization. Practitioners must emphasize the importance of regular dental check-ups and maintenance using 3D patient monitoring tools to evaluate the wear and morphological changes over time. Transillumination should be used to verify initial cracks, and static and dynamic occlusion should be monitored to avoid overloading due to excessive contact points.[15] Furthermore, it is important to continuously check the quality of the polished surface and re-polish it when necessary, in order to contribute to a better prognosis.

Clinicians must be aware of the gradual degradation that dental ceramics undergo over time due to oral conditions. Staying up-to-date with technological innovations, particularly understanding different toughness mechanisms, is vital for selecting the most appropriate ceramic resilience. Utilizing advanced CAD/CAM techniques ensures precise restorations and reduces premature failures. However, basic care, including regular dental check-ups and occlusion assessments, is equally important. Continuous education enables the implementation of best clinical practices.

In conclusion, the fatigue cycle is a significant concern that requires collaborative efforts from researchers, dental professionals and patients. By investing in research, education and preventive care, the dental community can lead the way for the development of more durable ceramic materials and ensure that patients continue to benefit
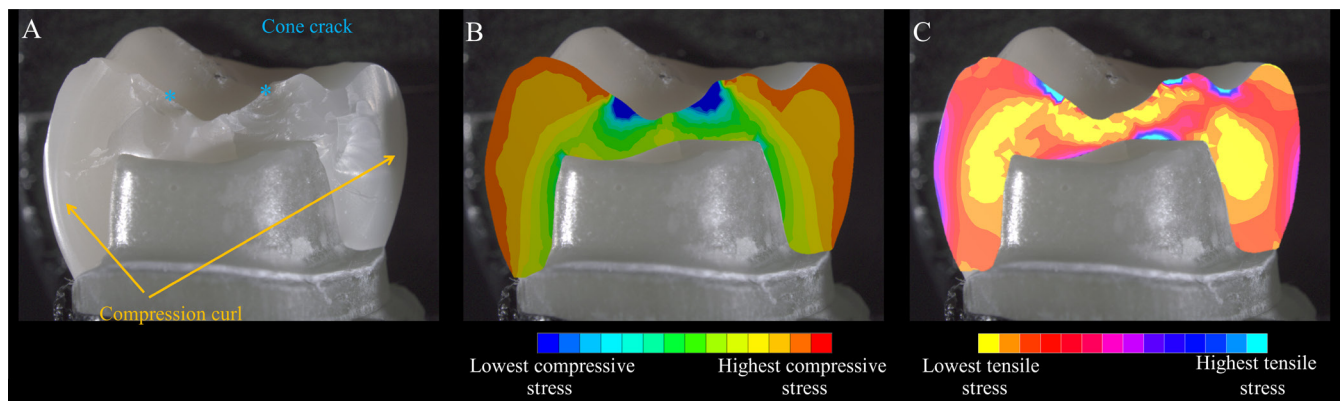
Fig. 1. Microscopy analysis of failed monolithic crown made of zirconia-reinforced lithium silicate after fatigue aging

A. Initial fractography in stereomicroscope from a fractured ceramic crown; B. Simulation showing the calculated compressive stress; C. Simulation showing the calculated tensile stress. In this example, fractography analysis was used with finite element analysis (FEA) to correlate the fracture features with the mechanical behavior during loading.

from their aesthetic and functional advantages. Together, we can address the challenges posed by ceramic fatigue and finally endorse reliable dental restorations.

ORCID iDs

João Paulo Mendes Tribst https://orcid.org/0000-0002-5412-3546
Luiz Felipe Valandro https://orcid.org/0000-0001-7203-6924
Amanda Maria de Oliveira Dal Piva
 https://orcid.org/0000-0002-3844-2053

References

1. El-Mowafy O, El-Aawar N, El-Mowafy N. Porcelain veneers: An update. *Dent Med Probl.* 2018;55(2):207–211. doi:10.17219/dmp/90729

2. Zhang Y, Sailer I, Lawn BR. Fatigue of dental ceramics. *J Dent.* 2013;41(12):1135–1147. doi:10.1016/j.jdent.2013.10.007

3. Alsarani M, De Souza G, Rizkalla A, El-Mowafy O. Influence of crown design and material on chipping-resistance of all-ceramic molar crowns: An in vitro study. *Dent Med Probl.* 2018;55(1):35–42. doi:10.17219/dmp/85000

4. Valandro LF, Cadore-Rodrigues AC, Dapieve KS, Machry RV, Rocha Pereira GK. A brief review on fatigue test of ceramic and some related matters in dentistry. *J Mech Behav Biomed Mater.* 2023;138:105607. doi:10.1016/j.jmbbm.2022.105607

5. Capobianco V, Baroudi K, Moraes Coelho Santos MJ, et al. Post-fatigue fracture load, stress concentration and mechanical properties of feldspathic, leucite- and lithium disilicate-reinforced glass ceramics. *Heliyon.* 2023;9(7):e17787. doi:10.1016/j.heliyon.2023.e17787

6. Aboushelib MN, Wang H, Kleverlaan CJ, Feilzer AJ. Fatigue behavior of zirconia under different loading conditions. *Dent Mater.* 2016;32(7):915–920. doi:10.1016/j.dental.2016.03.012

7. Lu Y, de Oliveira Dal Piva AM, Mendes Tribst JP, Feilzer AJ, Kleverlaan CJ. Does glaze firing affect the strength of advanced lithium disilicate after simulated defects? *Clin Oral Investig.* 2023;27(11):6429–6438. doi:10.1007/s00784-023-05246-1

8. de Carvalho Ramos N, Gullo Augusto M, Martins Alves LM, Kleverlaan CJ, de Oliveira Dal Piva AM. Wear of dental ceramics. *Braz Dent Sci.* 2023;26(1):e3638. doi:10.4322/bds.2023.3638

9. de Oliveira Dal Piva AM, Mendes Tribst JP, Benalcázar Jalkh EB, Costa Anami L, Bonfante EA, Bottino MA. Minimal tooth preparation for posterior monolithic ceramic crowns: Effect on the mechanical behavior, reliability and translucency. *Dent Mater.* 2021;37(3):e140–e150. doi:10.1016/j.dental.2020.11.001

10. Garbelotto LGD, Fukushima KA, Özcan M, Cesar PF, Volpato CAM. Chipping of veneering ceramic on a lithium disilicate anterior single crown: Description of repair method and a fractographic failure analysis. *J Esthet Restor Dent.* 2019;31(4):299–303. doi:10.1111/jerd.12468

11. Ozcan M, Valandro LF, Amaral R, Leite F, Bottino MA. Bond strength durability of a resin composite on a reinforced ceramic using various repair systems. *Dent Mater.* 2009;25(12):1477–1483. doi:10.1016/j.dental.2009.06.020

12. Monteiro Pereira R, Guimarães Ribas R, do Amaral Montanheiro TL, et al. An engineering perspective of ceramics applied in dental reconstructions. *J Appl Oral Sci.* 2023;31:e20220421. doi:10.1590/1678-7757-2022-0421

13. Oliveira Pilecco R, Saldanha da Rosa L, Rocha Pereira GK, Mendes Tribst JP, Gressler May L, Valandro LF. The loss of resin cement adhesion to ceramic influences the fatigue behavior of bonded lithium disilicate restorations. *J Mech Behav Biomed Mater.* 2023;148:106169. doi:10.1016/j.jmbbm.2023.106169

14. Scherrer SS, Lohbauer U, Bona AD, et al. ADM guidance-ceramics: Guidance to the use of fractography in failure analysis of brittle materials. *Dent Mater.* 2017;33(6):599–620. doi:10.1016/j.dental.2017.03.004

15. Gimenez-Gonzalez B, Setyo C, Gomez Picaza M, Mendes Tribst JP. Effect of defect size and tooth anatomy in the measurements of a 3D patient monitoring tool. *Heliyon.* 2022;8(12):e12103. doi:10.1016/j.heliyon.2022.e12103

Qualitative suggestions for the further development of the Standardized Tool for the Assessment of Bruxism (STAB)

Magdalini Thymi[1,A−D,F], Amir Farzan[1,A−D,F], Jari Ahlberg[2,E,F], Daniele Manfredini[3,E,F], Frank Lobbezoo[1,A,C,E,F]

[1] Department of Orofacial Pain and Dysfunction, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and Vrije Universiteit Amsterdam, the Netherlands

[2] Department of Oral and Maxillofacial Diseases, University of Helsinki, Head and Neck Center, Helsinki University Hospital, Finland

[3] Department of Medical Biotechnologies, School of Dentistry, University of Siena, 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

Address for correspondence
Frank Lobbezoo
E-mail: f.lobbezoo@acta.nl

Abstract

Background. The Standardized Tool for the Assessment of Bruxism (STAB) has recently been published. It contains Axis A for evaluating bruxism status and its potential consequences, and Axis B for bruxism risk, etiological factors and comorbid conditions. Suggestions from daily clinical practice can contribute to the further development of the STAB.

Objectives. The study aimed to investigate the experiences and attitudes of general dentists, dentists specialized in Orofacial Pain and Dysfunction (OPD) and Disability Care (DC) regarding the assessment and etiology of sleep and awake bruxism and to examine the concordance of their experiences and attitudes with the respective STAB axes and its domains.

Material and methods. Semi-structured interviews were conducted with 11 dentists. The main themes included bruxism assessment and etiology. Thematic analysis identified subthemes. The concordance between main themes, subthemes, and the corresponding STAB axes and domains was examined.

Results. Overall, there was a good correspondence between the experiences and attitudes of the interviewees and the respective STAB axes and domains. Some discrepancies were identified, but they were mainly related to the lack of appropriate tools for the DC setting.

Conclusions. It is suggested that future versions of the STAB include appropriate tools for the assessment of bruxism in DC settings.

Keywords: interview, bruxism, qualitative research

Introduction

Bruxism is defined as a masticatory muscle activity (MMA) with 2 distinct circadian manifestations: sleep bruxism, which occurs during sleep and is characterized as rhythmic (phasic) or non-rhythmic (tonic); and awake bruxism, which occurs during wakefulness and is characterized by repetitive or sustained tooth contact and/or by bracing or thrusting of the mandible.[1] In addition, a diagnostic grading system has been proposed for the assessment of bruxism by an international expert group and identified the entities of "possible", "probable" and "definite" sleep or awake bruxism.[1] The diagnosis of possible sleep and awake bruxism is based on a positive self-report. The probable sleep and awake bruxism diagnosis is based on a positive clinical inspection, with or without a positive self-report. The definite sleep and awake bruxism diagnosis is based on a positive instrumental assessment, with or without a positive self-report and/or a positive clinical inspection.[1,2] Clinicians usually base their bruxism assessments on self-reports and/or clinical examination.[3,4] Ideally, bruxism assessments in the clinic should be based on standardized tools.[5] The Standardized Tool for the Assessment of Bruxism (STAB) is a crucial part of the successful implementation of the bruxism diagnostic grading system,[5] allowing for consistent assessments in clinical practice and research. The investigation of which signs and symptoms are examined by clinicians in their daily practice may provide valuable suggestions for the design and further development of such a tool by ensuring that it captures clinically relevant domains.

The etiology of bruxism is a complex and highly debated issue. The evidence supports a pivotal role of the central and autonomic nervous systems in the regulation of both sleep and awake bruxism. This is in contrast to older theories which considered peripheral factors, such as occlusal and anatomical, to be important.[6] Over the years, a considerable number of other variables have been identified as risk factors for bruxism.[7] However, the exact etiology of sleep and awake bruxism remains unclear. A comprehensive assessment of factors presumed to be associated with bruxism is recommended to facilitate future research on the topic.[5] The experience gained by clinicians from their daily work may be used to better understand the etiology of bruxism and the assessment of etiological factors in the clinic.

Finally, it is important to emphasize the importance of bruxism in the field of special dental care. Although the available data is limited, bruxism appears to be highly prevalent in individuals with developmental disabilities (DD). For example, the prevalence rates are 42% in children with Down syndrome[8] and 69.4% in children with cerebral palsy.[9] At the same time, the assessment of bruxism can be challenging in these populations. Self-report instruments for the assessment of bruxism have not been validated in populations with DD, and clinicians and researchers often have to rely on clinical observation and/or proxy reports by the caregiver.[8–10] Overall, the assessment of bruxism in populations with DD is largely understudied.[11] These populations, however, should not be overlooked in studies on the design and further development of bruxism assessment tools.

A comprehensive STAB has recently been published.[5] The STAB consists of 2 main axes: an evaluation axis (Axis A), which consists of 3 assessment domains, namely subject-based, clinically-based and instrumentally-based assessments; and risk/etiological factors axis (Axis B) for the assessment of psychosocial factors, concurrent sleep and non-sleep conditions, drug and substance use or abuse, and additional factors.[5] In addition to the STAB, a brief bruxism screener (BruxScreen) was published. The BruxScreen is intended for use in general dental practices and large-scale epidemiological research projects, pending validity testing.[12] This study aimed to contribute to the further development of the recently published STAB by providing suggestions from daily clinical practice. More specifically, the objective of this study was to investigate the experiences and attitudes of general dentists as well as dentists specialized in Orofacial Pain and Dysfunction (OPD) and Disability Care (DC) regarding the assessment and etiology of sleep and awake bruxism. Additionally, this study aimed to examine the concordance of these experiences and attitudes with the axes and domains of the STAB.

Material and methods

Study design

A qualitative study was designed, and semi-structured interviews were conducted to address the aim of this study. Semi-structured interviews allow for the collection of a wide range of information about personal attitudes and experiences in the field of healthcare.[13] In this interview model, the interviewer specifies the topics through open-ended key questions. It allows the interviewee to discuss these subjects while also providing the opportunity to raise novel points that were not considered by the research team beforehand.[13] Thus, unique insights into the views, opinions, knowledge, and attitudes of clinicians can be collected.

Interviewee sampling

In this study, the purposive sampling method was employed to select the interviewees. Purposive sampling refers to the selection of interviewees based on specific criteria.[14] The determination of which criteria to adopt depends on a variety of factors, including the principal aims of the study.[14] This study aimed to contribute to the further development of the recently published STAB in general and specialized dental practices.[5] Therefore,

general dental practitioners were selected, as well as dentists specialized in fields where bruxism is an important part of daily practice, namely OPD and DC. General dentists with more than 2 years of experience and specialized dentists with their certificates approved by the relevant Dutch professional associations, i.e., the Dutch Association for Orofacial Pain and Prosthetic Dentistry (Nederlandse Vereniging voor Gnathologie en Prothetische Tandheelkunde (NVGPT)) for the OPD specialists, and the Association for the Promotion of Dental Healthcare for People with Disabilities (Vereniging Mondzorg voor Bijzondere Zorggroepen (VMBZ)) for the DC specialists, were included. The two-year criterion was chosen to ascertain experience in this field. Personal or professional affiliations between the authors and the interviewees were not an exclusion criterion. However, interviews were designed in such a way that each interviewer had no personal or professional affiliation with the person they interviewed.

To recruit participants, an advertisement was published on the LinkedIn page of the Department of OPD at the Academic Centre for Dentistry in Amsterdam (ACTA). Additionally, general dentists were approached through the personal networks of dentists who were employed at the Department of OPD. Specialized dentists were approached through the personal networks of the authors, as well as via the networks of the respective professional associations, i.e., NVGPT and VMBZ, after written permission from these associations had been obtained. The recruitment of participants took place from June to September 2020, with interviews conducted during the same period. The study was approved by the Ethics Committee of the ACTA (approval No. 2020219).

Interviewee background information

Prior to each interview, a digital questionnaire (https://www.qualtrics.com) was used to collect information about the participants' gender, number of years practicing dentistry, number of years as a specialized dentist, and postgraduate education in the field of bruxism completed within the past 5 years.

Interview method and data analysis

Two of the authors, AF ($n = 7$) and MT ($n = 4$), conducted the interviews. MT is a researcher and dentist with expertise in the fields of orofacial pain, oral movement disorders, tooth wear, and dental sleep medicine. The author has the experience in conducting qualitative research. AF is a sixth-year dental student with no experience in the field of qualitative research. Prior to conducting the first interview, an interview topic guide was designed based on the aims of this study, relevant scientific literature, the expertise and experience of 2 authors (MT and FL), and the results of pilot interviews. The topic guide served

as a memory assistant during the interview process.[14] The topic guide included 4 domains, i.e., assessment, etiology, consequences, and the treatment of bruxism. The domains of consequences and treatment were included for different purposes than the present study and will be presented in future publications.

Six pilot interviews were conducted. The first two interviews were conducted between 2 authors, MT and AF, with the objective of providing training to author AF. The latter 4 interviews were conducted between AF and dentists from the professional networks of the authors (AF and MT), both for training purposes and to further refine the topic guide. These 6 interviewees were not included in the final group of interviewees.

The setting for the interview was selected by the participating dentist and could be either physical or conducted via Skype video call (Microsoft Corporation, Redmond, USA). Each interview was allotted a time frame of up to 30 min. All interviews were audio-recorded using a tape recorder and subsequently transcribed verbatim by AF. During this process, any information that could potentially reveal the identity of the interviewee was removed. All transcriptions were reviewed by MT. The transcriptions were not returned to the interviewees for comments or corrections, and no interviews were repeated.

Thematic analysis was performed by AF and MT shortly after the acquisition of each interview. This analytical method was conducted in a series of steps, as outlined below, to identify subthemes for each main theme.[15] For this study, the main themes were predefined and included the assessment and etiology of bruxism.

In the first stage of the thematic analysis, the researchers identified and coded the initial themes by reviewing the transcribed interviews line by line. For each main theme, all initial themes that were conceptually related to one another were grouped into subthemes. Next, a thematic chart was constructed, in which the main themes were positioned in the top row. Below the main themes, each subtheme was assigned a single column, which contained all relevant textual data from the interviews. At the bottom of each column, the textual data was summarized according to the respective subtheme and, subsequently, per the main theme.[14]

The data was analyzed and the results were synthesized using ATLAS.ti (Scientific Software Development GmbH, Berlin, Germany) and Microsoft Excel (Microsoft Corporation) software. The coding and synthesis of the data were conducted by AF and MT independently, and any conflicts were resolved through discussion.

The interviewing process continued until information saturation was reached. This was the case when no new or secondary information was obtained from the data. Saturation was confirmed by 2 additional interviews.[14] After the thematic analysis was completed, the concordance between the main themes and subthemes from this study and the respective STAB axes and their domains was examined.

Results

This section presents the results of the thematic analysis of interviews and their concordance with the respective STAB axes and domains.

Interviewees

A total of 12 individuals were registered for participation in this study. One interviewee was unable to participate in the study due to unknown reasons. Consequently, 11 dentists participated in the study, of whom 7 were specialized dentists, and 4 were general dentists. The background information of the interviewees is presented in Table 1.

Thematic analysis

From the thematic analysis, 6 subthemes were obtained, 3 of which were related to the main theme of assessment (Table 2) and 3 to etiology (Table 3). All subthemes and items per subtheme are presented in the left columns of the tables. The concordance between the data derived from the interviews and the respective STAB items is presented. To facilitate a clear overview of this concordance, the STAB axes and domains are presented in the right columns of the tables.

Assessment

The main theme of assessment was subdivided into 3 categories, namely, anamnesis, clinical examination and additional diagnostics (Table 2).

Anamnesis

Topics reported by the interviewees in the anamnesis subtheme were related to the content of self-reports, challenges encountered during the anamnesis process and information from previous dental history, as described in the patient's health record (Table 2). Overall, there was a substantial degree of overlap between the content of these topics and Axis A of the STAB (Subject-Based Assessment (SBA)) (Table 2). However, certain issues emerged during the interviews that are not specifically addressed in the STAB. First, some interviewees described that self-reported awareness of bruxism is not a single time-point process. Rather, it can be a process that requires time in some cases, as individuals may become increasingly aware of their bruxism activity after their dentist has brought the issue to their attention. Second, it was reported that in individuals with communication disabilities, self-reporting may solely rely on proxy reports by caregivers. Third, some interviewees indicated that they use information from the patient's health record, such as reports of previous use of oral

Table 1. Characteristics of the interviewees

Characteristics	Values
Sample size, N	11
General dentists/specialized dentists*, n	4/7
Male/female, n	0/11
Practicing dentistry [years] M (range)	22 (6–38)
Being a specialized dentist [years] M (range)	10 (7–13)
Interviewees who attended a lecture on bruxism within the past 5 years, n	7
Interviewees who attended a congress on bruxism within the past 5 years, n	4
Interviewees who attended a course on bruxism within the past 5 years, n	1
Interviewees who read professional literature about bruxism within the past 5 years, n	9

* Orofacial Pain and Dysfunction (OPD) (n = 4) and Disability Care (DC) (n = 3); M – mean.

appliances. On the other hand, the questionnaires, which are mentioned as a means of current bruxism reporting on the STAB, were not mentioned by the interviewees.

Clinical examination

Topics that were reported in the clinical examination subtheme were grouped into 4 categories, namely extraoral signs, intraoral signs, pain, and others. A significant degree of overlap was observed with Axis A of the STAB (Clinically Based Assessment (CBA)) (Table 2). Two issues that emerged from the interviews but were not encountered in the STAB include the clinician's intuition, which refers to the situation in which clinicians partially rely on their intuition to guide them in the assessment of bruxism, and the issue of when to collect more information. In the course of the interviews, some respondents contended that in the absence of complaints in the history, no additional diagnostics are necessary.

Additional diagnostics

The additional diagnostics subtheme involves a discussion of imaging, specifically the acquisition of a panoramic radiograph to evaluate potential changes in the shape of the condylar bone, which could be indicative of bruxism. This topic is not addressed in the STAB. Moreover, a discrepancy between the findings of this study and the topics included in Axis A of the STAB (Instrumentally Based Assessment (IBA)) was found. The instrumental assessments proposed by the STAB for sleep bruxism, awake bruxism and additional instruments were not reported in the interviews of the present study.

Table 2. Thematic analysis of interviews for the assessment theme, and the comparison of interview results with Axis A of the Standardized Tool for the Assessment of Bruxism (STAB) (Assessment of Bruxism Status and Consequences)[5]

Study interviews		STAB Axis A	
subthemes	description	categories	domains
Anamnesis	Content of self-reports: – ask about complaints such as: headache, muscle cramps upon awakening, orofacial pain, grinding sounds, awareness of tooth wear – grinding sounds reported by caregiver in people with DD – grinding sounds reported by a bed partner	Subject-Based Assessment (SBA)	A1 (Sleep Bruxism Report) A2 (Awake Bruxism Report) A3 (Patient's Complaints)
	Challenges in anamnesis: – not a single time-point process: patients can become gradually aware of their bruxism activity – limited self-reporting in people with communication disabilities – validity of self-report		–
	Previous dental history: – bruxism-related complaints, as documented in the patient's health record – history of wearing a splint		A1 (Sleep Bruxism Report) A2 (Awake Bruxism Report)
Clinical examination	A. Extraoral signs Muscle and jaw volume: – voluminous masseter muscle – prominent jaws/jaw angles Temporomandibular joint sounds		A4 (Joints and Muscles)
	B. Intraoral signs Tooth wear: – wear facets – chipping of teeth – cervical lesions – fractures of teeth – fractures of restorations (fillings, crowns) – bruxopositions: pattern of antagonist teeth fitting exactly into each other – wear on splint – wear on removable denture Signs in soft tissues: – linea alba in the cheeks – tongue scalloping – red spots on the palate Endodontic treatment on one side	Clinically Based Assessment (CBA)	A5 (Intraoral and Extraoral Tissues) A6 (Teeth and Restorations)
	C. Pain No pain Dental pain Orofacial/TMD pain not related to the dentition Other issues, including difficulty in reporting pain in patients with disabilities		A4 (Joints and Muscles)
	D. Others Clinician's intuition/feeling When to perform more diagnostic procedures: – no complaint, no clinical examination and no treatment		–
Additional diagnostics	Imaging: – panoramic radiograph to evaluate the shape (changes) of the mandibular condyle –	Instrumentally Based Assessment (IBA)	– A7 (Sleep Bruxism) A8 (Awake Bruxism) A9 (Additional Instruments)

TMD – temporomandibular disorders; DD – developmental disabilities.

Etiology

The main theme of etiology was subdivided into 3 categories, namely psychosocial and behavioral factors, physical and dental factors, and the assessment of comorbidities (Table 3).

Psychosocial and behavioral factors

The interviewees acknowledged that stress was broadly associated with bruxism and it was also encountered in Axis B of the STAB (Psychosocial Assessment) (Table 3). Interestingly, the topic of processing or seeking stimuli arose from

the interviews, in which environmental over- or understimulation might be an etiological factor of bruxism in people with severe DD. Moreover, the interviews indicated that bruxism may be an acquired habit or occur in moments of concentration. These topics were not specifically addressed in the STAB. However, with regard to the psychological factors that may be related to bruxism, the STAB authors do mention that there may be many others, thus allowing space for topics such as those reported in the present interviews to be considered in the assessment of bruxism.

Physical and dental factors

Physical factors reported by the interviewees exhibited a high degree of concordance with Axis B of the STAB (Concurrent Non-Sleep Conditions Assessment and Prescribed Medications and Use of Substances Assessment). Discrepancies include the addressing of specific syndromes, such as Down syndrome and Rett syndrome, in the interviews, but not in the STAB. Moreover, there was some doubt expressed in the interviews as to

Table 3. Thematic analysis of interviews for the etiology theme, and the comparison of interview results with Axis B of the STAB (Risk and Etiological Factors and Comorbid Conditions)[5]

Study interviews		STAB Axis B
subthemes	description	
Psychosocial and behavioral factors	Psychosocial factors: – stress – life events – aggravation of spasticity in cerebral palsy – lack of awareness of stress	B1 (Psychosocial Assessment)
	Processing or seeking stimuli in severe DD: – environmental overstimulation: in people unable to communicate, bruxism may be the result of expressing the tension caused by overstimulation due to external stressors that cannot be communicated otherwise – environmental understimulation: seeking for stimulation through oral stimuli which are under one's own control due to a lack of stimuli from the environment	–
	Habits: – awake bruxism is a learned behavior, especially in individuals with DD	
	Concentration: – bruxism occurring during moments of concentration, which may not be immediately apparent	
Physical and dental factors	Age: – bruxism might be related to growth in children – age-related stress: the prevalence of bruxism is highest during the working years	
	Neurological disorders: – patients with cerebral palsy and spasticity present with severe bruxism – relationship between neurological disorders and bruxism remains uncertain	B3 (Concurrent Non-Sleep Conditions Assessment)
	Medication: – antidepressants – antipsychotics – polypharmacy – uncertain whether bruxism is related to medication	B4 (Prescribed Medications and Use of Substances Assessment)
	Substance use: – caffeine – smoking – drugs, particularly ecstasy, and previous drug addiction	
	Syndromes: – Down syndrome – Sanfilippo syndrome – Rett syndrome – Others	–
	Anatomical/occlusal factors: – do not play a role – less important than psychosocial factors – iatrogenic, i.e., restorations with occlusal contacts that are too high	
	–	B5 (Additional Factors Assessment)
Assessment of comorbidities	Differential diagnosis of other oral parafunctions: – biting on objects – tongue pressing	–
	Sleep bruxism comorbidities: – reflux – snoring, obstructive sleep apnea – sleepiness	B2 (Concurrent Sleep-Related Conditions Assessment)

whether neurological disorders are related to bruxism, even though such disorders are specifically mentioned in the STAB. Endocrine disorders and certain items in Axis B of the STAB (Additional Factors Assessment) were not mentioned at all in the interviews. Finally, dental factors, such as iatrogenic high occlusal contacts, were mentioned by the interviewees, but are not reported in the STAB (Table 3).

Assessment of comorbidities

The subjects discussed in the assessment of the comorbidities subtheme were related to the differential diagnosis of other oral parafunctions and the assessment of sleep bruxism comorbidities. More specifically, the interviewees argued that bruxism should be distinguished from other parafunctions with comparable consequences for the masticatory system, such as biting on objects and tongue pressing. This topic is not specifically addressed in the STAB. On the other hand, there was an overlap between the subject "sleep bruxism comorbidities", as it arose from the interviews, and Axis B of the STAB (Concurrent Sleep-Related Conditions Assessment) (Table 3).

Discussion

The present qualitative study aimed to contribute to the further development of the STAB by providing suggestions based on daily clinical practice. More specifically, the objective was to investigate the experiences and attitudes of general dentists, dentists specializing in OPD, and dentists specializing in DC regarding the assessment and etiology of sleep and awake bruxism and to examine the concordance of these experiences and attitudes with the respective axes of the STAB. Taken together, the items that emerged from the interviews had a high degree of overlap with those described in both axes of the STAB. The following sections will present the suggestions for each axis.

Axis A: Assessment of Bruxism Status and Consequences

Anamnesis

In the present study, interviewees reported that patients may become increasingly aware of their bruxism activity after discussing the topic with their dentist. To date, there is limited evidence to support this clinical observation. Kaplan and Ohrbach demonstrated that self-reporting of oral parafunctional behaviors, as assessed by the Oral Behaviors Checklist (OBC), exhibited substantial reliability over a 7-day period.[16] The study participants were made aware of their parafunctional behavior by being prompted

8 times per day to report their oral parafunctions, in accordance with the Ecological Momentary Assessment (EMA) paradigm.[16] On the other hand, with regard to sleep bruxism, it has been suggested that self-reports may be influenced by reporting bias, particularly in patients with painful temporomandibular disorders (TMD)[17] and non-painful jaw-muscle symptoms of the masticatory muscles.[18] However, longitudinal data on how both awake and sleep bruxism reporting can change over time under the influence of repeated assessments is generally lacking. One of the recommendations for the further development of the STAB would be to allow for repeated assessments of self-reported awake and sleep bruxism at certain intervals, such as regular dental check-ups. It is recommended that neutral questioning be employed, especially when assessing sleep bruxism in patients with TMD pain or other jaw-muscle symptoms,[17,18] in order to prevent reporting bias. As described below, instruments such as the BRUX scale[19] and the OBC[20] may prove valuable for this purpose.

Moreover, the present study demonstrated the necessity of proxy reports for individuals who are unable to self-report their bruxism activity due to certain disabilities. Interviewees in the present study described how caregivers of these individuals might report audible and visible sleep and awake bruxism activity and how, sometimes, this activity can be observed by dentists during dental appointments. However, to date, no instrument exists for the standardized assessment of such directly observed or proxy-reported bruxism activity. It is recommended that future studies work on the development of such an instrument, which may follow the paradigm of other fields, such as the assessment of pain based on the observation of behavior.[21,22] In the meantime, the developers of the STAB[5] and the BruxScreen[12] are encouraged to include a proxy-report field in these tools. A final remark on the topic of observing bruxism activity in a clinical setting is that this finding has implications for the bruxism diagnostic grading system.[23] Indeed, it may be argued that a definite awake bruxism diagnosis can be established if bruxism activity is directly observed, irrespective of instrumental assessments on which a definite diagnosis is currently based.[23]

Lastly, with regard to the anamnestic part of the bruxism assessment, the study showed that questionnaires were not employed as a means of assessing bruxism in the clinic. It remains plausible that such questionnaires are nevertheless used by some dentists, although they appear to be a relatively minor source of information in clinical practice. The use of diagnostic questionnaires as part of standard care is common in some tertiary clinical settings,[24] but it remains unknown whether encouraging the use of such questionnaires would be beneficial for general dental practices. Based on the results of the present study, it is recommended that future versions of the STAB provide a concise description of how these interviews and/or questionnaires should be implemented

330

M. Thymi et al. Qualitative suggestions for the STAB

in clinical practice. Currently, the OBC[20] is recommended by the Diagnostic Criteria for TMD (DC/TMD)[25] for the assessment of sleep and awake bruxism and is a freely available tool on the website of the International Network for Orofacial Pain and Related Disorders Methodology (INfORM).[26] The full OBC is included in the STAB. The recently developed BruxScreen[12] uses the BRUX scale, derived from the Oral Parafunctions Scale,[19] for the assessment of self-perceived clenching and grinding during wakefulness and sleep. Moreover, 2 additional questions were derived and slightly modified to assess the presence of light tooth contact and firm mandible bracing during wakefulness. The initial pilot testing of the BruxScreen in a population of dentists and patients at the University Clinics of Helsinki, Finland, and Sienna, Italy, yielded positive results regarding the comprehensiveness, feasibility and validity of the tool.[12] Thus, the BruxScreen is a promising tool for widespread use in regular dental care and large-scale epidemiological studies, provided further validity testing is completed.[12] The developers of the STAB and BruxScreen recommend performing validity testing in patient populations from all spectrums of abilities to ensure that no one is excluded based on disability.

Clinical examination

As shown in the Results section, there was a substantial degree of overlap between the topics reported by interviewees and those described in Axis A of the STAB (CBA). The assessment of bruxism based on intra- and extraoral clinical signs has also been described in other practice-based studies.[3,4] Recently, the BruxScreen presented a brief tool for the standardized assessment of extra- and intraoral signs that may be associated with bruxism.[12] Alongside the clinical signs that are assessed in the BruxScreen, the results of the present study provide suggestions for the development of a comprehensive instrument for the wider assessment of clinical signs of sleep and awake bruxism in future versions of the STAB.

Additional diagnostics

Discrepancies were identified between the additional diagnostic procedures described in the interviews of the present study and Axis A of the STAB (IBA). Radiographic evaluation of the condylar bone was reported in the present study, but not included in the STAB. Scientific evidence to support the radiographic features of the condylar bone as signs of bruxism is extremely scarce.[27–29] To date, it has not provided sufficient support for this notion. Other potential radiographic features of bruxism have been investigated in a very limited number of studies. Tassoker found no relationship between sleep bruxism and pulpal calcifications in a group of young women,[30] while Türp et al. observed increased bone apposition at the mandibular angles of adult bruxers

compared to an adolescent control group.[31] Consequently, no strong recommendations can be made regarding the integration of radiographic findings in the STAB, and further research on this topic is required. The use of instrumental approaches, such as EMA, electromyography (EMG) and polysomnography (PSG), was not reported by the interviewees. Ecological momentary assessment (also known as the experience sampling method (ESM)) is a technique that has been used in previous research settings.[32] Recently, a smartphone-based application that allows low-key, widespread implementation has been developed.[33] It is possible that the dentists in the present study were unaware of the existence of this application. Electromyography devices for the assessment of awake bruxism have been used in research settings,[34] and a significant variety of ambulatory EMG devices for the assessment of sleep bruxism exists.[35] However, to the best of the authors' knowledge, these devices are not available or feasible for regular care, at least not in the Netherlands, where the present study was performed. Moreover, it is necessary to achieve consensus on the most appropriate method to assess MMA using EMA methods and ambulatory EMG devices.[5,35] Further research on this topic is strongly recommended. It is also important to consider the commercial availability of smartphone applications and ambulatory EMG devices, as well as their accessibility for people with disabilities.

Axis B: Risk and Etiological Factors and Comorbid Conditions

Psychosocial and behavioral factors

In the present study, the participants acknowledged that psychological stress is associated with bruxism. However, they also reported that other factors, such as processing or seeking stimuli, habits and concentration, may play a role. The authors of the STAB aimed to evaluate the full spectrum of psychological and social factors that may be associated with bruxism.[5] In that context, the key question is: which of these psychological and social factors are associated with bruxism? It is beyond the scope of the present study to address this question. Instead, a recommendation will be made as to how to incorporate the views of the interviewed clinicians in future versions of the STAB.

In the interviews, clinicians referred to their patients experiencing and reporting stress mainly in the context of daily stress and life events. The interviewers did not perform further probing to specify the concept of stress, which represents a limitation of the present study. In scientific literature, psychological stress is reported to occur when environmental demands challenge or exceed an individual's adaptive capacity, as perceived by the individual.[36] Stress can be expressed through a wide spectrum

of adverse emotional states, including depression, anxiety, distress, and lack of well-being.[36] The translation of this knowledge into daily practice necessitates the use of appropriate tools and questionnaires to assess the emotional states. Examples of such tools are the Generalized Anxiety Disorder-7 (GAD-7)[37] to evaluate anxiety, and the Patient Health Questionnaire-9 (PHQ-9)[38] to evaluate depression. These questionnaires are already incorporated into the DC/TMD evaluation process. Their incorporation into the STAB (the PHQ-9 is already part of the ToolKit of the STAB) could be considered to promote homogeneity of measurements in populations of patients who are assessed for the presence of TMD and bruxism. The inclusion of the brief PHQ-4 for the assessment of anxiety and depression, which is currently included in the STAB, will possibly hamper such homogeneity of measurements. For populations in which stress cannot be assessed through questionnaires due to certain disabilities,[39] it is necessary to include appropriate tools in the STAB, e.g., the Disability Distress Assessment Tool (DisDAT).[40]

What is more, clinical reports of bruxism being associated with states of concentration, and, in individuals with DD, with overstimulation or seeking of stimuli, require further research. Regarding the topic of concentration, evidence for an association with bruxism is extremely limited. In a study by Major et al., sleep bruxers were found to have no greater mental or physical alertness than the control group.[41] Conversely, other masticatory muscle activities, such as chewing, have been shown to be positively associated with attention.[42] Based on the findings of the present study, no specific recommendations can be made regarding the incorporation of concentration into the STAB.

With regard to the topic of stimuli processing, the authors are unaware of any evidence that would suggest an association with bruxism. However, studies can be found on the interplay between stimuli processing and oral function. Little et al. observed distinct sensory processing patterns, i.e., responses to environmental stimuli, such as avoiding and seeking behavior, in children with autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) compared to children with typical development (TD).[43] In the same study, the authors examined the differential processing of sensory stimuli across various modalities, including auditory, visual, tactile, etc. Significant differences were observed in the processing of oral stimuli in children with ASD and ADHD compared to children with TD.[43] These differences in sensory processing may be associated with eating difficulties,[43,44] although a possible association with sleep and/or awake bruxism is unknown. Moreover, Kirby et al. studied sensory interests, repetitions, and seeking (SIRS) behaviors, i.e., behaviors that occur in interaction with sensory stimuli.[45] The authors of this study found that children with ASD displayed significantly more SIRS behaviors than children with other

DD or TD.[45] However, no significant differences were observed between children with ASD, DD and TD in terms of oral SIRS behaviors of mouthing (bringing objects to the open mouth, placing them in the mouth and/or licking them) and biting (biting objects with teeth).[45] Based on the findings of the present study and relevant literature, no specific recommendation can be made regarding the topic of stimuli processing and the STAB. However, it is recommended that further studies investigate the extent to which bruxism activity occurs as a reaction to environmental stimuli, taking into account differences in sensory processing. The findings of such studies may shed light on the etiology of bruxism and could be incorporated into future versions of the STAB.

Physical and dental factors

The present study showed a high degree of overlap between the physical factors reported by the interviewees and Axis B of the STAB (Concurrent Non-Sleep Conditions Assessment and the Prescribed Medications and Use of Substances Assessment). Specific syndromes, such as Down syndrome and Rett syndrome, were mentioned by the interviewees, yet they are not included in the STAB. Indeed, a high prevalence of bruxism has been reported for these syndromes,[8,46,47] but the prevalence, etiology, consequences, and treatment of sleep and awake bruxism in these populations are largely understudied. To facilitate research and clinical care in populations with DD and neurodevelopmental conditions, it is recommended that these conditions be added to the STAB.

Finally, regarding the dental factors, the present study identified iatrogenic high occlusal contacts as a potential etiological factor for bruxism activity. However, the literature does not support a causal association between occlusal factors and bruxism.[6] Therefore, it is not recommended to introduce these factors into the STAB.

Assessment of comorbidities

In the present interviews, it was argued that other oral parafunctions should be assessed and distinguished from bruxism. Another clinical practice-based study revealed that only 39.1% of respondents considered other oral parafunctions when assessing and interviewing a patient with suspected bruxism.[3] The STAB conceptualizes the assessment of concurrent non-sleep conditions, which may be associated with increased MMA.[5] Oral parafunctions could be a source of loading for the masticatory system, with consequences similar to those of bruxism, such as temporomandibular disorder (TMD) pain.[24] Therefore, it is recommended that oral parafunctions be included in the differential diagnostic procedure when assessing an individual with suspected bruxism. For this purpose, the STAB has adopted the OBC.[20] Furthermore, comorbid conditions with sleep bruxism, such as reflux,

snoring, obstructive sleep apnea, and sleepiness, were described in the interviews. These are the items that are also described in Axis B of the STAB (Concurrent Sleep-Related Conditions Assessment),[5] and to some extent in the practice-based study by Guillot et al.[3]

Conclusions

The findings of this study indicate a high degree of concordance between the experiences and attitudes of general dentists and dentists specialized in OPD and DC regarding the diagnosis and etiology of sleep and awake bruxism, and both axes of the STAB. This concordance indicates that the proposed STAB generally captures the items that dentists deem relevant to their clinical practice. Nevertheless, some issues were identified with regard to both STAB axes. The main issue involved the lack of appropriate tools for the DC setting. Based on the study results, the recommendations for the further development of the STAB, derived from interviews with 11 dentists, are summarized as follows:

1. Ensure that the STAB includes appropriate tools for the assessment of bruxism and related factors in individuals across the entire spectrum of abilities;
2. Allow for repeated assessment of self-reported awake and sleep bruxism at certain intervals, with brief, neutral questioning;
3. Allow for standardized assessment of directly observed or proxy-reported bruxism activity;
4. Provide a concise description of the methodology for implementing self-report interviews and/or questionnaires in clinical practice;
5. Develop a standardized tool for the wider assessment of clinical signs of bruxism;
6. Provide guidance on the use of instrumental approaches for the assessment of bruxism, with a particular focus on the scoring of MMA;
7. Include DD and neurodevelopmental conditions in the assessment of concurrent non-sleep conditions.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Academic Centre for Dentistry in Amsterdam (ACTA) (approval No. 2020219).

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

Magdalini Thymi https://orcid.org/0000-0002-8544-6495
Amir Farzan https://orcid.org/0000-0002-7360-5452
Jari Ahlberg https://orcid.org/0000-0002-6052-0441
Daniele Manfredini https://orcid.org/0000-0002-4352-3085
Frank Lobbezoo https://orcid.org/0000-0001-9877-7640

References

1. Lobbezoo F, Ahlberg J, Raphael KG, et al. International consensus on the assessment of bruxism: Report of a work in progress. *J Oral Rehabil*. 2018;45(11):837–844. doi:10.1111/joor.12663
2. Manfredini D, Ahlberg J, Wetselaar P, Svensson P, Lobbezoo F. The bruxism construct: From cut-off points to a continuum spectrum. *J Oral Rehabil*. 2019;46(11):991–997. doi:10.1111/joor.12833
3. Guillot M, Jungo S, Maniere A, Laplanche O, Tillier Y, Ehrmann E. Diagnosis and management of bruxism: Evaluation of clinical practices in France. *Cranio*. 2019;39(5):412–423. doi:10.1080/08869634.2019.1661657
4. Thymi M, Rollman A, Visscher CM, Wismeijer D, Lobbezoo F. Experience with bruxism in the everyday oral implantology practice in the Netherlands: A qualitative study. *BDJ Open*. 2018;4:17040. doi:10.1038/s41405-018-0006-4
5. Manfredini D, Ahlberg J, Aarab G, et al. Standardised Tool for the Assessment of Bruxism. *J Oral Rehabil*. 2024;51(1):29–58. doi:10.1111/joor.13411
6. Klasser GD, Rei N, Lavigne GJ. Sleep bruxism etiology: The evolution of a changing paradigm. *J Can Dent Assoc*. 2015;81:f2. PMID:25633110.
7. Kuhn M, Türp JC. Risk factors for bruxism. *Swiss Dent J*. 2018;128(2):118–124. PMID:29533049.
8. López-Pérez R, López-Morales P, Borges-Yáñez SA, Maupomé G, Parés-Vidrio G. Prevalence of bruxism among Mexican children with Down syndrome. *Downs Syndr Res Pract*. 2007;12(1):45–49. doi:10.3104/reports.1995
9. Peres ACD, Ribeiro MO, Juliano Y, César MF, de Almeida Santos RC. Occurrence of bruxism in a sample of Brazilian children with cerebral palsy. *Spec Care Dentist*. 2007;27(2):73–76. doi:10.1111/j.1754-4505.2007.tb00332.x
10. Avcu N, Ozbek M, Kurtoglu D, Kurtoglu E, Kansu O, Kansu H. Oral findings and health status among hospitalized patients with physical disabilities, aged 60 or above. *Arch Gerontol Geriatr*. 2005;41(1):69–79. doi:10.1016/j.archger.2004.11.005
11. Lang R, White PJ, Machalicek W, et al. Treatment of bruxism in individuals with developmental disabilities: A systematic review. *Res Dev Disabil*. 2009;30(5):809–818. doi:10.1016/j.ridd.2008.12.006
12. Lobbezoo F, Ahlberg J, Verhoeff MC, et al. The bruxism screener (BruxScreen): Development, pilot testing and face validity. *J Oral Rehabil*. 2024;51(1):59–66. doi:10.1111/joor.13442
13. Gill P, Stewart K, Treasure E, Chadwick B. Methods of data collection in qualitative research: Interviews and focus groups. *Br Dent J*. 2008;204(6):291–295. doi:10.1038/bdj.2008.192
14. Ritchie J, Lewis J, eds. Qualitative Research Practice: A Guide for Social Science Students and Researchers. 1st ed. London, UK: SAGE Publications; 2003.
15. Stewart K, Gill P, Chadwick B, Treasure E. Qualitative research in dentistry. *Br Dent J*. 2008;204(5):235–239. doi:10.1038/bdj.2008.149
16. Kaplan SEF, Ohrbach R. Self-report of waking-state oral parafunctional behaviors in the natural environment. *J Oral Facial Pain Headache*. 2016;30(2):107–119. doi:10.11607/ofph.1592
17. Raphael KG, Janal MN, Sirois D, et al. Validity of self-reported sleep bruxism among myofascial temporomandibular disorder patients and controls. *J Oral Rehabil*. 2015;42(10):751–758. doi:10.1111/joor.12310
18. Thymi M, Shimada A, Lobbezoo F, Svensson P. Clinical jaw-muscle symptoms in a group of probable sleep bruxers. *J Dent*. 2019;85:81–87. doi:10.1016/j.jdent.2019.05.016
19. van der Meulen MJ, Lobbezoo F, Aartman IHA, Naeije M. Self-reported oral parafunctions and pain intensity in temporomandibular disorder patients. *J Orofac Pain*. 2006;20(1):31–35. PMID:16483018.
20. Ohrbach R, Markiewicz MR, McCall WD. Waking-state oral parafunctional behaviors: Specificity and validity as assessed by electromyography. *Eur J Oral Sci*. 2008;116(5):438–444. doi:10.1111/j.1600-0722.2008.00560.x

21. Corbett A, Achterberg W, Husebo B, et al. An international road map to improve pain assessment in people with impaired cognition: The development of the Pain Assessment in Impaired Cognition (PAIC) meta-tool. *BMC Neurol*. 2014;14:229. doi:10.1186/S12883-014-0229-5

22. de Vries MW, Visscher C, Delwel S, et al. Orofacial pain during mastication in people with dementia: Reliability testing of the orofacial pain scale for non-verbal individuals. *Behav Neurol*. 2016;2016:3123402. doi:10.1155/2016/3123402

23. Lobbezoo F, Aarab G, Koyano K, Manfredini D. Assessment of sleep bruxism. In: Kryger M, Roth T, Goldstein CA, eds. *Principles and Practice of Sleep Medicine*, 7th ed. Philadelphia, PA: Elsevier; 2022:1636–1644.

24. van Selms MK, Visscher CM, Knibbe W, Thymi M, Lobbezoo F. The association between self-reported awake oral behaviors and orofacial pain depends on the belief of patients that these behaviors are harmful to the jaw. *J Oral Facial Pain Headache*. 2020;34(3):273–280. doi:10.11607/ofph.2629

25. Schiffman E, Ohrbach R, Truelove E, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: Recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Group†. *J Oral Facial Pain Headache*. 2014;28(1):6–27. doi:10.1007/BF00338494

26. International Network for Orofacial Pain and Related Disorders Methodology (INfORM). The Oral Behavior Checklist. https://inform-iadr.com/wp-content/uploads/2024/03/Oral-Behaviors-Checklist_English-7-19-08-checkboxes.pdf. Accessed November 11, 2021.

27. Eninanç İ, Yalçın Yeler D, Çınar Z. Investigation of mandibular fractal dimension on digital panoramic radiographs in bruxist individuals. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2021;131(5):600–609. doi:10.1016/j.oooo.2021.01.017

28. Gulec M, Tassoker M, Ozcan S, Orhan K. Evaluation of the mandibular trabecular bone in patients with bruxism using fractal analysis. *Oral Radiol*. 2021;37(1):36–45. doi:10.1007/s11282-020-00422-5

29. Padmaja Satheeswarakumar L, Elenjickal TJ, Ram SKM, Thangasamy K. Assessment of mandibular surface area changes in bruxers versus controls on panoramic radiographic images: A case control study. *Open Dent J*. 2018;12:753–761. doi:10.2174/1745017901814010753

30. Tassoker M. Evaluation of the relationship between sleep bruxism and pulpal calcifications in young women: A clinico-radiological study. *Imaging Sci Dent*. 2018;48(4):277–281. doi:10.5624/ISD.2018.48.4.277

31. Türp JC, Simonek M, Dagassan D. Bone apposition at the mandibular angles as a radiological sign of bruxism: A retrospective study. *BMC Oral Health*. 2021;21(1):537. doi:10.1186/s12903-021-01804-9

32. Chen CY, Palla S, Erni S, Sieber M, Gallo LM. Nonfunctional tooth contact in healthy controls and patients with myogenous facial pain. *J Orofac Pain*. 2007;21(3):185–193. PMID:17717957.

33. Manfredini D, Bracci A, Djukic G. BruxApp: The ecological momentary assessment of awake bruxism. *Minerva Stomatol*. 2016;65(4):252–255. PMID:27374364.

34. Prasad S, Paulin M, Cannon RD, Palla S, Farella M. Smartphone-assisted monitoring of masticatory muscle activity in freely moving individuals. *Clin Oral Investig*. 2019;23(9):3601–3611. doi:10.1007/s00784-018-2785-3

35. Thymi M, Lobbezoo F, Aarab G, et al. Signal acquisition and analysis of ambulatory electromyographic recordings for the assessment of sleep bruxism: A scoping review. *J Oral Rehabil*. 2021;48(7):846–871. doi:10.1111/joor.13170

36. Cohen S, Janicki-Deverts D, Miller GE. Psychological stress and disease. *JAMA*. 2007;298(14):1685–1687. doi:10.1001/jama.298.14.1685

37. Spitzer RL, Kroenke K, Williams JBW, Löwe B. A brief measure for assessing generalized anxiety disorder: The GAD-7. *Arch Intern Med*. 2006;166(10):1092–1097. doi:10.1001/archinte.166.10.1092

38. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: Validity of a brief depression severity measure. *J Gen Intern Med*. 2001;16(9):606–613. doi:10.1046/j.1525-1497.2001.016009606.x

39. Kıldahl AN, Helverschou SB, Bakken TL, Oddli HW. "If we do not look for it, we do not see it": Clinicians' experiences and understanding of identifying post-traumatic stress disorder in adults with autism and intellectual disability. *J Appl Res Intellect Disabil*. 2020;33(5):1119–1132. doi:10.1111/jar.12734

40. Regnard C, Reynolds J, Watson B, Matthews D, Gibson L, Clarke C. Understanding distress in people with severe communication difficulties: Developing and assessing the Disability Distress Assessment Tool (DisDAT). *J Intellect Disabil Res*. 2007;51(Pt 4):277–292. doi:10.1111/j.1365-2788.2006.00875.x

41. Major M, Rompré PH, Guitard F, et al. A controlled daytime challenge of motor performance and vigilance in sleep bruxers. *J Dent Res*. 1999;78(11):1754–1762. doi:10.1177/00220345990780111301

42. Hirano Y, Onozuka M. Chewing and attention: A positive effect on sustained attention. *Biomed Res Int*. 2015;2015:367026. doi:10.1155/2015/367026

43. Little LM, Dean E, Tomchek S, Dunn W. Sensory processing patterns in autism, attention deficit hyperactivity disorder, and typical development. *Phys Occup Ther Pediatr*. 2018;38(3):243–254. doi:10.1080/01942638.2017.1390809

44. Nadon G, Feldman DE, Dunn W, Gisel E. Association of sensory processing and eating problems in children with autism spectrum disorders. *Autism Res Treat*. 2011;2011:541926. doi:10.1155/2011/541926

45. Kirby AV, Little LM, Schultz B, Baranek GT. Observational characterization of sensory interests, repetitions, and seeking behaviors. *Am J Occup Ther*. 2015;69(3):6903220010p1-9. doi:10.5014/ajot.2015.015081

46. Bianco E, Rota D. Oral findings in Rett syndrome: An update and review of the literature. *Dent Med Probl*. 2018;55(4):441–445. doi:10.17219/dmp/99203

47. Lai YYL, Downs J, Zafar S, Wong K, Walsh L, Leonard H. Oral health care and service utilisation in individuals with Rett syndrome: An international cross-sectional study. *J Intellect Disabil Res*. 2021;65(6):561–576. doi:10.1111/jir.12834

Correlation between the expression of the *iNOS*, *caspase-3* and *a-SMA* genes in the parotid glands of albino rats following the administration of two antihistamines from two different generations

Dina Rady[1,B–F], Marwa Magdy Abbass[1,A,B,E,F], Heba Hakam[1,A,B,E,F], Rasha Rady[2,E], Iman Aboushady[3,B–F]

[1] Department of Oral Biology, Faculty of Dentistry, Cairo University, Egypt

[2] Department of Pediatrics, Faculty of Medicine, Cairo University, Egypt

[3] Department of Oral Biology, Faculty of Dentistry, Modern University For Technology and Information, Cairo, Egypt

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

Abstract

Background. Several medications, including antihistamines, can alter salivary gland function, causing dry mouth or xerostomia. Antihistamines are commonly used for treating allergic rhinitis.

Objectives. The aim of the present study was to compare and correlate the effects of first-generation vs. second-generation H1-antihistamines on the parotid glands of rats.

Material and methods. Twelve adult male albino rats were used; 4 rats served as a control group (group I) and the remaining rats were divided into 2 groups: group II received promethazine hydrochloride; and group III received cetirizine dihydrochloride for 3 weeks. The parotid salivary glands were dissected, and examined histologically and analyzed histomorphometrically for the acinar area percentage. In addition, mRNA gene expression of *iNOS*, *caspase-3* and *a-SMA* was assessed using quantitative real-time polymerase chain reaction (qRT-PCR). Finally, all the obtained data was statistically analyzed.

Results. Histologically, group I showed the typical architecture of the gland. In group II, degenerative changes were noticed, including acinar degeneration and shrinkage with widened connective tissue septa, intracellular vacuolization, and increased inflammatory cell infiltration. In group III, similar histological features were detected as in group II, but to a lesser extent. Histomorphometric results revealed significant differences in the acinar area percentage between various groups. In addition, qRT-PCR results showed a significant increase in *iNOS* expression in both groups II and III as compared to group I, *caspase-3* gene expression was significantly increased in group II, while in group III, it increased non-significantly. Finally, *a-SMA* gene expression non-significantly decreased in both groups II and III. A significant positive correlation was observed between *caspase-3* and *iNOS* gene expression, while an inverse correlation was noticed between *caspase-3* and *a-SMA* gene expression.

Conclusions. The administration of antihistamines resulted in changes in the rat salivary glands, which could be due to the induction of oxidative stress and the resultant apoptotic effect. These changes were suggested to occur mainly through action on muscarinic receptors; yet, action on histamine receptors could not be excluded. However; these effects were less marked with the second-generation antihistamine.

Keywords: nitric oxide synthase, caspase 3, alpha smooth muscle actin, histamine antagonists

Introduction

Xerostomia is a medical condition characterized by oral cavity dryness. It has a multifactorial etiology; most commonly, it is due to systemic diseases like Sjögren's syndrome, head and neck region radiation and the administration of xerostomia-causing medications.[1] Various drugs, including antihistamines, can alter salivary gland function, causing oral dryness, which depends greatly on the number and dosage of the drugs being administered.[2] The incidence of xerostomia is growing because of the aging population, as older individuals commonly take several medications.[3] The occurrence of xerostomia is accompanied by a greater prevalence of fungal infections and dental caries, in addition to difficulties in speech, mastication and deglutition, which consequently affects the patient's oral health and compromise their quality of life.[1]

H1-antihistamines are among the most common medications used worldwide. They are structurally unrelated to histamine and act as inverse agonists by stabilizing its inactive conformation.[4] Moreover, they are functionally classified into 2 broad groups, being first-generation or classical sedating group and the second-generation or non-sedating group.[5]

First-generation H1-receptor antagonists (e.g., promethazine hydrochloride) are lipophilic and can cross the blood–brain barrier. Consequently, they can affect the central histaminic receptors, so they are known for their sedating effect.[5] Furthermore, promethazine has strong anticholinergic properties, facilitated by blocking the responses to acetylcholine mediated by muscarinic receptors. This atropine-like effect [Please confirm.] is accountable for the most common side effects of antihistamine medicines, including xerostomia (dry mouth), observed in their clinical usage.[6]

Conversely, second-generation antagonists, piperazine derivatives and carboxylated metabolites of hydroxyzine, were developed with more selectivity to peripheral H1-receptors, and with a very poor affinity for muscarinic, adrenergic or serotonergic receptors. Also, second-generation H1-antihistamines can be considered relatively non-sedating, with no anticholinergic effects, as they poorly penetrate through the blood–brain barrier, and are less likely to affect the central histaminic cholinergic or adrenergic receptors.[5]

The antihistaminic activity of cetirizine, a second-generation H1-receptor antagonist, has been reviewed previously, showing no measurable affinity for receptors other than H1 in vitro, in addition to negligible anticholinergic activity in animal models. However, clinically, dry mouth was documented with cetirizine more commonly than in the case of placebo.[7]

The third-generation antihistamines, active metabolites of second-generation agents, possess many of the desirable clinical effects of the first-generation agents with a more tolerable side effect profile.[8]

Despite the negative impact of antihistamines on salivary secretion, which has been documented clinically, the molecular mechanism behind this effect is yet unknown. To better understand the cellular and molecular basis of xerostomia, the current study compared the effects of long-term administration of promethazine hydrochloride, a first-generation H1-antihistamine, and cetirizine dihydrochloride, a second-generation antihistamine.

Material and methods

Drugs

The following drugs were used in the study:
– Histaloc®: 25 mg of promethazine hydrochloride (first-generation H1-receptor antagonist) (Julphar Gulf Pharmaceutical Industries, Ras Al Khaimah, UAE); and
– Zyrtec®: 10 mg of cetirizine dihydrochloride (second-generation H1 -eceptor antagonist) (GlaxoSmithKline, Al Haraneyah, Egypt).

Animals

Based on a previous study conducted by Shahabooei et al., the average difference in the alveolar bone healing between 2 experimental groups of rats was 4 ±1.3%.[9] Using a power of 80%, 3 rats were needed in each group. This number had been increased to 4 in each group to compensate for losses during breeding. Sample size calculation was performed using PS: Power and Sample Size Calculation program, v. 3.1.2 (Vanderbilt University, Nashville, USA).

Twelve adult male albino rats (*Rattus norvegicus albinus*, Wistar strain), weighing approx. 150–200 g, were obtained and bred at the Faculty of Medicine, Cairo University, Egypt. They were kept in individual cages, with a light/dark cycle of 12 h, fed a standardized diet and given water ad libitum.

Experimental design

The animals were randomly assigned into 3 groups using the Random Sequence Generator program (https://www.random.org/sequences). Four rats served as a control group (group I), where the rats received distilled water daily via oral gavage for 3 successive weeks.

The remaining rats served as experimental groups, and they were randomly divided into 2 groups, 4 rats in each. Groups II and III included rats that received 4.8 mg/kg promethazine hydrochloride and 3 mg/kg cetirizine dihydrochloride, respectively, in distilled water daily via oral gavage for 3 successive weeks.

Investigations

After 3 weeks, rats from all studied groups were euthanized via an intracardiac overdose of sodium thiopental

(80 mg/kg). The parotid salivary glands were dissected and investigated. The assessment steps were carried out by blinded investigators.

Light microscopy examination

In the Department of Oral Biology, Faculty of Dentistry, Cairo University, the samples were fixed in 10% buffered formalin for 48 h, dehydrated in ethyl alcohol, cleared in xylol, and embedded in paraffin wax (El Gomhoureya for Drugs Trade and Medical Supplies, Cairo, Egypt). Sections of 4–6 μm were cut, mounted on glass slides, stained with the hematoxylin and eosin (H&E) stain, and then examined under a light microscope (Leica, St. Gallen, Switzerland), using magnifications ×200 and ×400.

Histomorphometric analysis

The acinar percentage area was assessed in photomicrographs at magnification ×400. Image analysis was done with the use of the ImageJ software, v. 1.53d (https://imagej.net/ij). Subsequently, the H&E images were converted by splitting the channels to the requisite color and the thresholds were adjusted specifically for the acinar area percentage. The dimensions were converted from pixels to millimeters by the software. Four non-overlapping microscopic fields were randomly selected and evaluated.

qRT-PCR analysis

In the Microbiology Unit, Department of Biochemistry, Faculty of Medicine, Cairo University, a total RNA isolation kit (Qiagen, Germantown, USA) was used to isolate total RNA from the obtained samples following the manufacturer's instructions. According to the kit protocol, RNA extracted from the specimens was reverse transcribed using a cDNA Reverse Transcription kit (Fermentas, Waltham, USA). cDNA was then amplified and analyzed using the StepOne™ software, v. 3.1 (Applied Biosystems, Waltham, USA). Using the comparative ΔΔCT method, relative mRNA gene expression was standardized with regard to the mean critical threshold values of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), a housekeeping gene (control gene). The sequences of the applied primers (ThermoFisher Scientific, USA) for inducible nitric oxide synthase (*iNOS*), caspase 3 (*caspase-3*), alpha smooth muscle actin (*α-SMA*), and *GAPDH* genes are listed in Table 1.

Statistical analysis

All data from histomorphometric analysis and qRT-PCR analysis was coded and entered using IBM SPSS Statistics for Windows, v. 25 (IBM Corp., Armonk, USA). Numerical data was summarized and expressed as mean and standard deviation (*M* ±*SD*). Comparisons

Table 1. Primer sequences of all studied genes

Gene symbol	Primer sequence (from 5' to 3')	Accession number
iNOS	F: 5'-GTTCCCCCAGCGGAGCGATG-3' R: 5'-ACTCGAGGCCACCCACCTCC-3'	NM_012611.3
caspase-3	F: 5'CTGGACTGCGGTATTGAG-3' R: 5'-GGGTGCGGTAGAGTAAGC-3'	NM_053304.1
α-SMA	F: 5'-CCGACCGAATGCAGAAGGA-3' R: 5'-ACAGAGTATTTGCGCTCCGAA-3'	NM_001613.4
GAPDH	F: 5'-CCATTCTTCCACCTTTGATGCT-3' R: 5'-TGTTGCTGTAGCCATATTCATTGT-3'	NM_017008.4

between the studied groups were performed using the analysis of variance (ANOVA) with multiple comparisons of Tukey's post hoc tests. The level of significance was set at $p < 0.05$. Correlations between quantitative variables (*iNOS*, *caspase 3* and *α-SMA*) were investigated using Pearson's correlation coefficient.[10]

Results

Light microscopy results

Group I

The histological examination of the parotid salivary gland tissues of the control group revealed normal glandular structure. Serous acini were closely packed together with intercalated and striated ducts in glandular lobules, with narrow normal connective tissue septa in between. Small to medium-sized blood vessels could be observed close to striated and excretory ducts (Fig. 1).
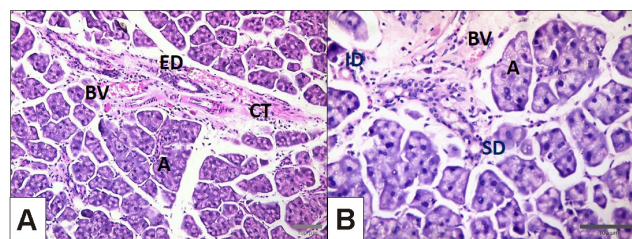
Fig. 1. Photomicrographs of the parotid gland tissues of the control group (group I)

A – normal histological structure, with closely packed acini (A) and connective tissue septa (CT) displaying a blood vessel containing RBCs (BV), as well as an excretory duct (ED) with normal lining; B – serous acini (A) with well-defined outlines, intralobular ducts (intercalated ducts (ID) and striated ducts (SD)) with normal lining, and small blood vessels (BV). RBCs – red blood cells. Scale bar – 100 μm.

Group II

The shrinkage of serous acini with widened connective tissue septa in between could be observed in most areas of the gland. Intracytoplasmic vacuolization was obvious in serous acini and the lining of some striated ducts. In addition, many atrophied and degenerated acini were observed.

Regarding excretory ducts, some of them displayed areas of degeneration and discontinuity in their lining. The degenerated acini were replaced with marked chronic inflammatory cell infiltration in discrete gland regions. Moreover, rare blood vessels were seen near striated ducts, and in connective tissue septa, some blood vessels were dilated, with areas of discontinuity in their endothelial lining, and contained few red blood cells (RBCs) in their lumen (Fig. 2).
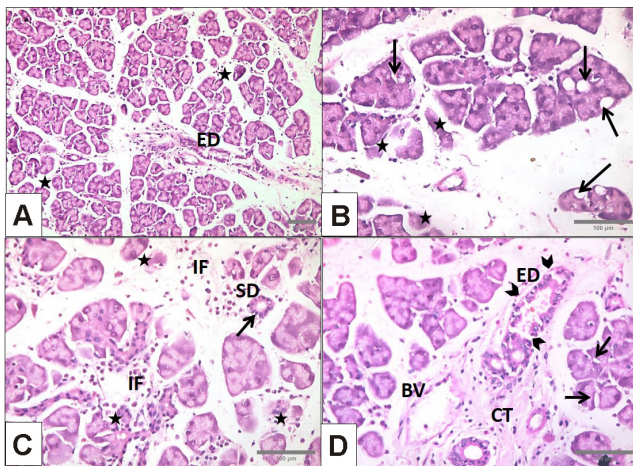
Fig. 2. Photomicrographs of the parotid gland tissues of the Histaloc group (group II)

A – shrinkage of serous acini with widened interlobular spaces (asterisks) and an excretory duct (ED) in connective tissue septa; B – intracytoplasmic vacuolization among serous acini (arrows) and numerous degenerated acini (asterisks); C – degenerated acini (asterisks) with massive chronic inflammatory cell infiltrate (IF) replacing the acinar portions, and cytoplasmic vacuolization in the striated duct (SD) lining (arrow); D – connective tissue septa (CT) displaying an excretory duct (ED) with discontinuity in its lining (arrow heads), and a dilated blood vessel (BV) with discontinuity in its endothelial lining, intracytoplasmic vacuolization (arrows). Scale bar – 100 μm.

Group III

The examination of sections obtained from this group showed enhanced histological features when compared to group II. This was evidenced by the presence of less degenerative changes among serous acini, which were closely packed in many areas of the gland, with narrow connective tissue septa in between. Less intracytoplasmic vacuolization could be detected in acinar cells and some of the ductal cells. Moreover, fewer atrophied and degenerated acini were observed. Intralobular ducts were easily detected with their normal lining and were frequently associated with small blood vessels engorged with RBCs. Connective tissue septa showed few inflammatory cell infiltrates, while excretory ducts showed normal lining in most areas of the gland and congested blood vessels with almost normal lining (Fig. 3).

Histomorphometric results

Regarding the acinar area percentage, statistically significant differences were observed between the studied groups ($p = 0.021$) (Table 2, Fig. 4). The lowest acinar
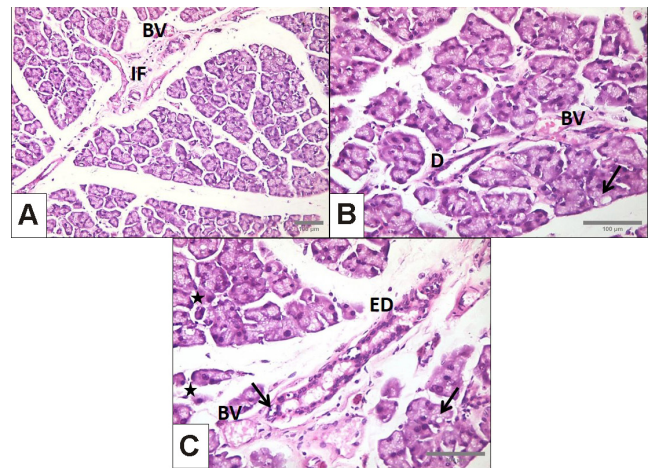
Fig. 3. Photomicrographs of the parotid gland tissues of the Zyrtec group (group III)

A – closely packed serous acini (A) connective tissue septa displaying few inflammatory cell infiltrates (IF), an excretory duct (ED) with normal lining, and a blood vessel (BV) engorged with RBCs; B – acinar vacuolization (arrow), intralobular duct (D) with normal lining, closely related to blood vessels (BV) engorged with RBCs; C – few intracytoplasmic vacuoles in the acinar and ductal cells (arrows), scattered degenerated acini (asterisks), and connective tissue septa displaying an excretory duct (ED) with continuous lining and congested blood vessels (BV) with RBCs. Scale bar – 100 μm.

Table 2. Results of ANOVA for the acinar area percentage [%] in all studied groups

Group	$M \pm SD$	SE	F	p-value
Group I	53.34 ±9.06	4.53		
Group II	35.95 ±2.51[a]	1.26	6.15	0.021*
Group III	46.82 ±7.88	3.94		

* statistically significant; [a] significant difference vs. group I.
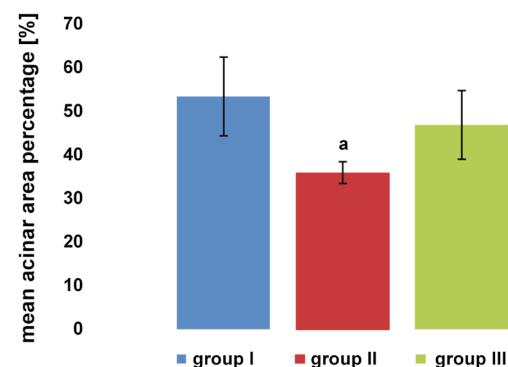
Fig. 4. Column chart comparing the mean acinar area percentage among all studied groups

The letter 'a' denotes a significant difference vs. group I.

percentage area was detected in group II, while the highest was recorded in group I. In addition, a statistically significant decrease in the acinar percentage area was observed in group II when compared to group I ($p = 0.017$). However, the acinar percentage area in group III was decreased as compared to group I and was

increased as compared to group II, although these results did not reach statistical significance ($p = 0.432$ and $p = 0.133$, respectively).

qRT-PCR results

Statistically significant differences were demonstrated between the studied groups regarding *iNOS*, *caspase-3* and *α-SMA* gene expression ($p < 0.001$, $p < 0.001$ and $p = 0.002$, respectively) (Table 3).

Table 3. Results of ANOVA for the expression of the studied genes [AU] relative to *GADPH* in all studied groups

Gene expression	Group I	Group II	Group III	*p*-value
iNOS	4.68 ±0.12	5.49 ±0.21[a]	5.33 ±0.2[a]	<0.001*
caspase-3	4.31 ±0.11	6.43 ±0.41[a]	4.58 ±0.28[b]	<0.001*
α-SMA	5.09 ±0.14	4.75 ±0.18	5.41 ±0.21[b]	0.002*

* statistically significant; [a] significant difference vs. group I; [b] significant difference vs. group II.

Regarding *iNOS* gene expression, a statistically significant increase was detected in both groups II and III as compared to group I ($p < 0.001$ and $p = 0.002$, respectively), while a decrease was detected in group III as compared to group II ($p = 0.412$). Conversely, *caspase-3* gene expression showed a statistically significant increase in group II as compared to group I ($p < 0.001$), while a non-significant increase was observed in group III as compared to group I ($p = 0.423$). However, *caspase-3* gene expression in group III was significantly decreased when compared to group II ($p < 0.001$). Finally, *α-SMA* gene expression was decreased in both groups II and III as compared to group I, although this did not reach statistical significance ($p = 0.056$ and $p = 0.071$, respectively), while a statistically significant increase in its expression was observed in group III when compared to group II ($p = 0.001$) (Fig. 5).
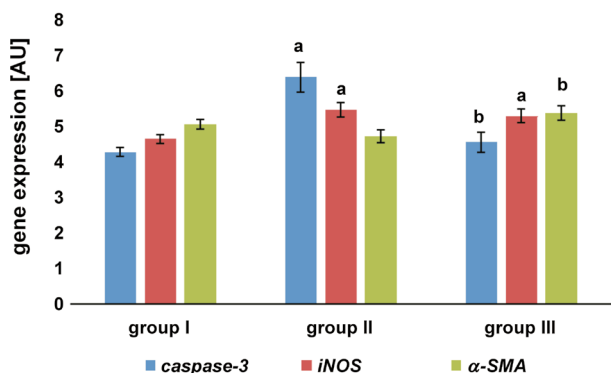
Fig. 5. Column chart comparing the mean expression of different genes among all studied groups

The letter 'a' denotes a significant difference vs. group I, whereas the letter 'b' denotes a significant difference vs. group II.

A significant positive correlation was observed between *caspase-3* and *iNOS* gene expression ($p = 0.046$; $r = 0.585$) (Table 4, Fig. 6). Conversely, the comparison between *caspase-3* and *α-SMA* gene expression revealed a significant inverse correlation ($p = 0.018$; $r = -0.666$) (Table 4, Fig. 7). Finally, no association was found between iNOS and α-SMA gene expression ($p = 0.721$; $r = -0.115$) (Table 4).

Table 4. Correlation between the expression of the studied genes

Gene expression		*iNOS*	*caspase-3*	*α-SMA*
iNOS	*r*	1	0.585	−0.115
	p-value	–	0.046*	0.721
	n	12	12	12
caspase-3	*r*	0.585	1	−0.666
	p-value	0.046*	–	0.018*
	n	12	12	12
α-SMA	*r*	−0.115	−0.666	1
	p-value	0.721	0.018*	–
	n	12	12	12

* correlation is significant at the level of $p < 0.05$ (2-tailed).
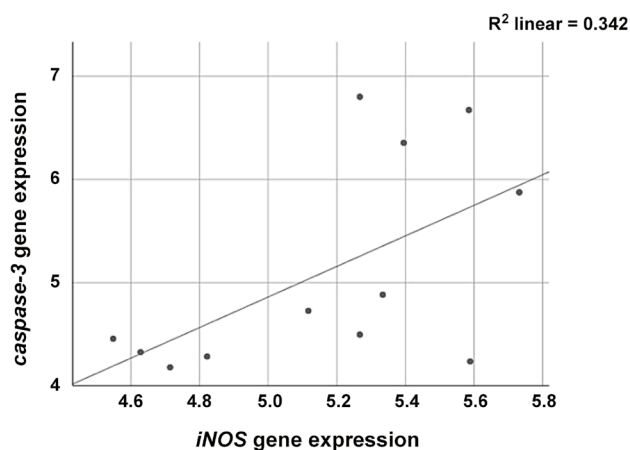
Fig. 6. Scatter chart showing a significant positive correlation between *caspase-3* and *iNOS* gene expression ($p < 0.046$; $r < 0.585$)
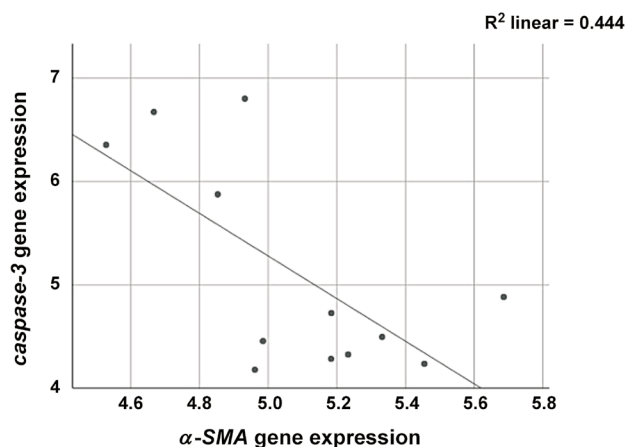
Fig. 7. Scatter chart showing a significant inverse correlation between *caspase-3* and *α-SMA* gene expression ($p < 0.018$; $r < -0.666$)

Discussion

Several medications can cause xerostomia as a common side effect, including β blockers, antipsychotics, antidepressants, and antihistamines.[2] Older generations of H1-antihistamines penetrate the blood–brain barrier, thus causing sedation, while second-generation H1-antihistaminic drugs are considered safer, resulting in fewer side effects.[4]

In the literature, cetirizine has been authorized as a potent second-generation H1-antihistamine and a first-choice antihistamine for treating allergic diseases. Cetirizine is normally used with daily dosing, as it is documented to be safe and well-tolerated.[11] On the other hand, promethazine is a first-generation H1-antihistamine that possesses anticholinergic properties causing xerostomia and other complications, such as blurred vision, dry nasal passages, dilated pupils, constipation, and urinary retention, and the American Geriatrics Society (AGS) categorizes it as a potentially inappropriate drug for the elderly.[12]

While the adverse effect of antihistamines on salivary secretion has been reported clinically, the underlying cellular mechanism remains unclear. Therefore, the current work investigated the effects of long-term administration of a first-generation H1-antihistamine, promethazine hydrochloride, as compared to second-generation cetirizine dihydrochloride, on the rat's parotid glands to clarify the underlying mechanism at the cellular and molecular levels.

The three-week duration of this study was selected to investigate the effects of long-term administration of both drugs. It has been previously established that in adult rats, every day of the animal's life is approx. equivalent to 34.8 human days (i.e., 1 rat month is comparable to 3 human years).[13]

Following the administration of antihistamines, the following changes were observed in the rat parotid glands: shrinkage and degeneration of serous acini; intracellular vacuolization in acini and the duct system; congested blood vessels; and increased inflammatory cell infiltration. These changes were more prominent following treatment with promethazine hydrochloride (group II) when compared to the cetirizine dihydrochloride-treated group (group III). In addition, histomorphometric results showed that the acinar percentage area was the lowest in group II.

Coinciding with the present findings, similar age-related degenerative changes have been observed in human labial salivary glands, as demonstrated by the presence of acinar atrophy and fibrosis, together with a diffuse inflammatory cell infiltrate.[14] In addition, Tatar et al. demonstrated acinar and ductal vacuolization in the rat's submandibular salivary glands following the administration of an antihistamine, namely desloratadine.[15] More recently, Mohammed et al. histologically evaluated the rat's parotid salivary gland tissues following treatment with desloratadine.[16] The authors demonstrated remark-able cytoplasmic vacuolization and the atrophy of serous acini, as well as increased interstitial spaces between parenchymal elements and mononuclear cell infiltrations.[16] The existence of vacuolization and consequent alteration in the content of secretory granules is associated with diminished salivary secretion.[17] Similarly, Choi et al. reported diminished salivary flow rates in association with atrophy and vacuolization in the salivary glands.[18]

The demonstrated histological changes following the administration of antihistamines could be attributed to the induction of oxidative stress. This assumption was based on previous findings by Tatar et al., who linked the effect of an antihistamine, desloratadine, with the induced mitochondrial oxidative stress and the resultant intra-mitochondrial reactive oxygen species (ROS),[15] thus bringing an equilibrium between oxidants and antioxidants.[19] Reactive oxygen species have various physiological roles and are normally created as by-products resulting from oxygen metabolism.[20] The nitric oxide synthase (NOS) family is significantly important in different physiological and pathological processes. Three isoforms for NOSs have been recognized, namely endothelial, inducible and neuronal NOSs (eNOS, iNOS and nNOS, respectively).[21] Generally, eNOS and nNOS are constantly generated at low concentrations by relevant cells; however, iNOS, the inducible type, has an important role in immunity and inflammation. This isoform is enhanced in inflammation, leading to exacerbated inflammatory effects.[22] In the present study, *iNOS* gene expression was significantly increased in both antihistamine groups as compared to the control group. Moreover, this expression was non-significantly decreased in group III when compared to group II. These results support the histological alterations observed in the antihistamine-treated groups (groups II and III), which were more obvious in group II rather than in group III.

Excess cellular ROS could damage proteins, lipids, nucleic acids, and membranes, eventually activating cell death processes, such as apoptosis. Apoptosis is a tightly controlled process necessary for the survival of living organisms.[23] Apoptotic caspases include upstream initiators (caspases 2, 8, 9, and 10) and downstream effectors (caspases 3, 6 and 7).[24] Herein, the gene expression of *caspase-3* was significantly increased in the promethazine hydrochloride group (group II) as compared to the untreated control, while it was non-significantly increased in the cetirizine dihydrochloride group (group III) as compared to the control group. This could be related to the observations made by Olianas et al., who investigated the effects of an acetylcholine receptor agonist, carbachol, on the interferon-β-induced apoptosis of a human neuroblastoma cell line.[25] The authors found that carbachol inhibited mitochondrial cytochrome c release, the activation of caspases 3, 7 and 9, as well as DNA destruction. They added that this antiapoptotic effect of carbachol was mediated through M3 muscarinic receptors.[25]

Based on these findings, it could be deduced that muscarinic receptor antagonists, like antihistamines, may be involved in enhancing apoptosis.

Our findings further support several recent in vitro and clinical studies confirming the pro-apoptotic and antitumor properties of antihistamines, with regard to both first- and second-generation drugs. For example, a first-generation antihistamine, cyproheptadine, caused a dose-dependent elevation in apoptosis in the C6 glioblastoma cell line, suggesting a potential anticancer effect of this drug.[26] In addition, second-generation drugs, clemastine and desloratadine, induced apoptosis in cutaneous T cell lymphoma cell lines.[27] In the present investigation, *caspase-3* gene expression was significantly decreased in group III when compared to group II. Thus, it could be deduced that promethazine hydrochloride exerted a more pronounced apoptotic effect than cetirizine dihydrochloride, which may be related to enhanced *iNOS* gene expression in the promethazine hydrochloride-treated group. This assumption was based on the correlation results of the current study, which revealed a significant positive correlation between the gene expression of *iNOS* and *caspase-3*.

This positive correlation could be attributed to the association between antihistamine-induced apoptosis and enhanced oxidative stress. For example, Trybus et al. demonstrated that azelastine, a second-generation antihistamine, induced a significant concentration-dependent increase in cytoplasmic vacuoles, an increase in ROS, increased oxidative stress, and DNA damage, and obviously promoted apoptosis in human cervical adenocarcinoma cells through the stimulation of caspases 3 and 7, and the inhibition of Bcl-2 protein.[28] The authors suggested the contribution of ROS in causing DNA damage, which may, in turn, trigger apoptosis.[28]

The muscarinic M3 receptor was assumed to be related to smooth muscle mass development, as M3 receptor-deficient mice displayed lower levels of alpha smooth muscle actin in airway arteries and bronchial tissues.[29] In normal salivary glands, *α-SMA* is expressed by the periacinar myoepithelial cells, in salivary ducts and normal blood vessels.[30] Herein, we revealed a non-significant decrease in *α-SMA* gene expression in the antihistamine groups II and III as compared to the control group. However, the regulatory effect of cetirizine dihydrochloride on *α-SMA* expression was significantly less than that of promethazine hydrochloride. These findings could be attributed to antagonizing the action of histamine, which is essential for *α-SMA* expression. The importance of histamine in *α-SMA* expression was demonstrated by Tibbo et al., who evidenced a significant reduction in α-SMA at both the protein and gene expression levels, following the treatment of a rabbit model of arthrofibrosis with ketotifen, a second-generation antihistamine, when compared to the untreated control group.[31]

In our study, the correlation analysis of *caspase-3* and *α-SMA* gene expression revealed a significant inverse relationship. This finding supports the demonstrated pro-apoptotic role of antihistamines in the present work, and the decreased *α-SMA* expression could result from the apoptosis of myoepithelial cells. Additionally, since the M3 receptor has been suggested to be critical in smooth muscle mass development[30] and consequent *α-SMA* expression, antihistamines as potential antimuscarinics acting on M3 receptors could result in decreased *α-SMA* expression. Since myoepithelial cells are essential for conveying saliva from the acini and supporting the parenchyma during secretion, any alterations in these cells could lead to defective salivary secretion, eventually causing xerostomia.

Conversely, we found no association between *α-SMA* and *iNOS* gene expression in the current work. On the contrary, Brennan et al. reported that a significant positive correlation existed between *iNOS* and *α-SMA* gene expression in pleomorphic adenoma cases.[30] However, this difference could be attributed to the difference in tissue types, as we investigated this correlation in normal and not cancerous salivary glands.

The more advanced degenerative effects in the first-generation (promethazine hydrochloride) group in comparison with the second-generation (cetirizine dihydrochloride) one in the present work agree with a previous study that compared both generations.[32] That study demonstrated that older antihistamines resulted in prominent side effects in the central nervous system (CNS) in addition to anticholinergic effects, including xerostomia, while the newly developed antihistamines (H1 receptor-specific antagonists) were related to a significantly lower incidence of xerostomia.[32] Similarly, Liu and Farley, demonstrated that second-generation antihistamines were more selective for histamine than first-generation drugs, with cetirizine having the highest potency toward histamine receptors.[33] Besides, cetirizine did not affect the concentration–response relationship for acetylcholine, suggesting a minimum or lack of interaction with M3 receptors. This could clarify why in our study, cetirizine dihydrochloride, as a second-generation antihistamine, exerted a less inhibitory effect on *α-SMA* gene expression than a first-generation antihistamine, promethazine hydrochloride. Despite the muscarinic-dependent xerostomic effect of antihistamines,[33] this effect could also be mediated through H1 receptors present in the salivary glands. This could explain why the new-generation antihistamines, with very low antimuscarinic effects, could still lead to xerostomia via their antagonistic effect on H1 receptors.[12]

Conclusions

We conclude that the administration of both first- and second-generation antihistamines induced several degenerative alterations in the rat parotid salivary glands,

342

D. Rady et al. Effect of antihistamines on the parotid gland

which could be mediated through enhanced oxidative stress, with subsequent increased apoptosis and diminished *α-SMA* gene expression, eventually leading to xerostomia, a well-known adverse effect of antihistamines. However, these effects were more marked following the administration of the first-generation rather than the second-generation antihistamine. Despite the unfavorable risk–benefit profile of first-generation antihistamines, they continue to be overconsumed because of their over-the-counter status and long history of use. Additionally, the significant pro-apoptotic effect of first-generation H1 receptor antagonists is promising in treating cancer diseases, particularly salivary gland carcinomas. Within the limitation of the current study, it is suggested to monitor the evidence of the superior safety of new-generation antihistamines. Thus, third-generation antihistamines are recommended to be investigated in future studies, using a bigger sample size.

Ethics approval and consent to participate

The experiment was carried out according to the recommendations of the ethics committee on animal experimentation at the Institutional Animal Care and Use Committee (IACUC), Cairo University, Egypt. This research was done in compliance with the ARRIVE guidelines and regulations (https://arriveguidelines.org).

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

Dina Rady https://orcid.org/0000-0002-9672-6935
Marwa Magdy Abbass https://orcid.org/0000-0002-6455-7516
Heba Hakam https://orcid.org/0000-0002-2427-1695
Rasha Rady https://orcid.org/0000-0003-1155-6306
Iman Aboushady https://orcid.org/0000-0003-3284-1878

References

1. Kapourani A, Kontogiannopoulos KN, Manioudaki AE, et al. A review on xerostomia and its various management strategies: The role of advanced polymeric materials in the treatment approaches. *Polymers (Basel)*. 2022;14(5):850. doi:10.3390/polym14050850
2. Wolff A, Joshi RK, Ekström J, et al. A guide to medications inducing salivary gland dysfunction, xerostomia, and subjective sialorrhea: A systematic review sponsored by the World Workshop on Oral Medicine VI. *Drugs R D*. 2017;17(1):1–28. doi:10.1007/s40268-016-0153-9
3. Anil S, Vellappally S, Hashem M, Preethanath RS, Patil S, Samaranayake LP. Xerostomia in geriatric patients: A burgeoning global concern. *J Investig Clin Dent*. 2016;7(1):5–12. doi:10.1111/jicd.12120
4. Church MK, Church DS. Pharmacology of antihistamines. *Indian J Dermatol*. 2013;58(3):219–224. doi:10.4103/0019-5154.110832
5. Van Schoor J. Antihistamines: A brief review. *Prof Nurs Today*. 2012;16(5):16–21. https://hdl.handle.net/10520/EJC127059. Accessed September 4, 2022.
6. Cantisani C, Ricci S, Grieco T, et al. Topical promethazine side effects: Our experience and review of the literature. *Biomed Res Int*. 2013;2013:151509. doi:10.1155/2013/151509
7. Portnoy JM, Dinakar C. Review of cetirizine hydrochloride for the treatment of allergic disorders. *Expert Opin Pharmacother*. 2004;5(1):125–135. doi:10.1517/14656566.5.1.125
8. Ellis AK, Day JH. Second-and third-generation antihistamines in the treatment of urticaria. *Dermatol Ther*. 2008. doi:10.1046/j.1529-8019.2000.00034.x
9. Shahabooei M, Razavi SM, Minaiyan M, et al. A histomorphometric study of the effect of doxycycline and erythromycin on bone formation in dental alveolar socket of rat. *Adv Biomed Res*. 2015;4:71. doi:10.4103/2277-9175.153895
10. Schober P, Boer C, Schwarte LA. Correlation coefficients: Appropriate use and interpretation. *Anesth Analg*. 2018;126(5):1763–1768. doi:10.1213/ANE.0000000000002864
11. Corsico AG, Leonardi S, Licari A, et al. Focus on the cetirizine use in clinical practice: A reappraisal 30 years later. *Multidiscip Respir Med*. 2019;14:40. doi:10.1186/s40248-019-0203-6
12. Kim JH, Park SH, Moon YW, et al. Histamine H1 receptor induces cytosolic calcium increase and aquaporin translocation in human salivary gland cells. *J Pharmacol Exp Ther*. 2009;330(2):403–412. doi:10.1124/jpet.109.153023
13. Sengupta P. The laboratory rat: Relating its age with human's. *Int J Prev Med*. 2013;4(6):624–630. PMID:23930179. PMCID:PMC3733029.
14. Sørensen CE, Larsen JO, Reibel J, et al. Associations between xerostomia, histopathological alterations, and autonomic innervation of labial salivary glands in men in late midlife. *Exp Gerontol*. 2014;57:211–217. doi:10.1016/j.exger.2014.06.004
15. Tatar A, Parlak SN, Yayla M, Ugan RA, Polat E, Halici Z. Effects of allergic rhinitis and desloratadine on the submandibular gland in a rat allergy model. *Int Forum Allergy Rhinol*. 2015;5(12):1164–1169. doi:10.1002/alr.21589
16. Mohammed IJ, Sarhat ER, Wadee SA, Al-Shiakhani SM. Histological and biochemical evaluation of the effect of desloratadine drug in parotid gland tissues. *Anb Med J*. 2021;17(2):72–77. doi:10.33091/amj.2022.171074
17. Şimşek G, Gürocak S, Karadağ N, et al. Protective effects of resveratrol on salivary gland damage induced by total body irradiation in rats. *Laryngoscope*. 2012;122(12):2743–2748. doi:10.1002/lary.23609
18. Choi JS, Park IS, Kim SK, Lim JY, Kim YM. Analysis of age-related changes in the functional morphologies of salivary glands in mice. *Arch Oral Biol*. 2013;58(11):1635–1642. doi:10.1016/j.archoralbio.2013.07.008
19. Sarhat ER. Altered serum marker of thyroid profile and antioxidant enzymes in individuals with Alzheimer's disease. *Int Res J Pharm*. 2019;10(1):56–60. doi:10.7897/2230-8407.100110
20. Pizzino G, Irrera N, Cucinotta M, et al. Oxidative stress: Harms and benefits for human health. *Oxid Med Cell Longev*. 2017;2017:8416763. doi:10.1155/2017/8416763
21. Costa ED, Rezende BA, Cortes SF, Lemos VS. Neuronal nitric oxide synthase in vascular physiology and diseases. *Front Physiol*. 2016;7:206. doi:10.3389/fphys.2016.00206
22. Papi S, Ahmadizar F, Hasanvand A. The role of nitric oxide in inflammation and oxidative stress. *Immunopathol Persa*. 2019;5(1):e08. doi:10.15171/ipp.2019.08
23. Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim Biophys Acta*. 2016;1863(12):2977–2992. doi:10.1016/j.bbamcr.2016.09.012
24. Vakifahmetoglu-Norberg H, Norberg E, Perdomo AB, et al. Caspase-2 promotes cytoskeleton protein degradation during apoptotic cell death. *Cell Death Dis*. 2013;4(12):e940. doi:10.1038/cddis.2013.463
25. Olianas MC, Dedoni S, Onali P. Protection from interferon-β-induced neuronal apoptosis through stimulation of muscarinic acetylcholine receptors coupled to ERK1/2 activation. *Br J Pharmacol*. 2016;173(19):2910–2928. doi:10.1111/bph.13570

26. Kacar S, Hacioglu C, Kar F, Sahinturk V, Kanbak G. Cyproheptadine causes apoptosis and decreases inflammation by disrupting thiol/disulfide balance and enhancing the levels of SIRT1 in C6 glioblastoma cells. *Toxicol In Vitro.* 2021;73:105135. doi:10.1016/j.tiv.2021.105135

27. Döbbeling U, Waeckerle-Men Y, Zabel F, Graf N, Kündig TM, Johansen P. The antihistamines clemastine and desloratadine inhibit STAT3 and c-Myc activities and induce apoptosis in cutaneous T-cell lymphoma cell lines. *Exp Dermatol.* 2013;22(2):119–124. doi:10.1111/exd.12086

28. Trybus E, Król T, Trybus W. The multidirectional effect of azelastine hydrochloride on cervical cancer cells. *Int J Mol Sci.* 2022;23(11):5890. doi:10.3390/ijms23115890

29. Kistemaker LE, Bos ST, Mudde WM, et al. Muscarinic M_3 receptors contribute to allergen-induced airway remodeling in mice. *Am J Respir Cell Mol Biol.* 2014;50(4):690–698. doi:10.1165/rcmb.2013-0220OC

30. Brennan PA, Umar T, Zaki GA, et al. Are myoepithelial cells responsible for the widespread expression of inducible nitric oxide synthase in pleomorphic adenoma? An immunohistochemical study. *J Oral Pathol Med.* 2000;29(6):279–283. doi:10.1034/j.1600-0714.2000.290607.x

31. Tibbo ME, Limberg AK, Salib CG, et al. Anti-fibrotic effects of the antihistamine ketotifen in a rabbit model of arthrofibrosis. *Bone Joint Res.* 2020;9(6):302–310. doi:10.1302/2046-3758.96.BJR-2019-0272.R2

32. Scully C, Bagan JV. Adverse drug reactions in the orofacial region. *Crit Rev Oral Biol Med.* 2004;15(4):221–239. doi:10.1177/154411130401500405

33. Liu H, Farley JM. Effects of first and second generation antihistamines on muscarinic induced mucus gland cell ion transport. *BMC Pharmacol.* 2005;5:8. doi:10.1186/1471-2210-5-8

Comparative evaluation of the remineralizing potential of *Salvadora persica* and probiotic yogurt on incipient enamel lesions: An ex-vivo study

Noura Mohammed Bakr[1,A,D,F], Abeer Abdelmaksoud Mohamed[2,B,C,F], Ghada Abdelwahab Salem[3,4,C–F]

[1] Department of Oral and Dental Biology, Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt

[2] Department of Oral Biology, Faculty of Dentistry, Fayoum University, Cairo, Egypt

[3] Department of Orthodontics and Pediatric Dentistry, Institute of Oral and Dental Research, National Research Centre, Cairo, Egypt

[4] Department of Orthodontics and Pedodontics, Faculty of Oral and Dental Medicine, Nahda University in Beni Suef, Egypt

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

Address for correspondence
Ghada Abdelwahab Salem
E-mail: ga.salem@nrc.sci.eg

Abstract

Background. *Salvadora persica* (miswak) is known to exert antibacterial, antifungal, antioxidant, and anticariogenic effects by elevating the pH of plaque after the consumption of sucrose.

Objectives. The study aimed to compare the effectiveness of *S. persica* and probiotic yogurt in the remineralization of tooth enamel on artificially produced enamel lesions.

Material and methods. A total of 40 intact human premolars were collected and each tooth was sectioned longitudinally into 2 identical halves in a buccolingual direction. The buccal halves were selected for inclusion in this study, and standardized windows (5 mm × 3 mm) were isolated on the buccal surface of the enamel. The samples were incubated in a demineralizing solution at 37°C for 96 h. Subsequently, they were randomly selected for treatment with one of the experimental remineralizing solutions (*S. persica* or probiotic yogurt). After treatment, the samples were examined using scanning electron microscopy (SEM), energy dispersive X-ray (EDX) and polarized light microscopy at baseline, after demineralization and after remineralization.

Results. The remineralizing effect of *S. persica* was found to be greater than that of probiotic yogurt. With regard to mineral content, *S. persica* exhibited the highest calcium and phosphorus levels among all groups. No significant differences were observed between the samples treated with *S. persica* and normal enamel.

Conclusions. *Salvadora persica* extract has been demonstrated to effectively reduce the demineralization of enamel in experimental conditions. Furthermore, it has the potential to restore the mineral content to its original level.

Keywords: scanning electron microscopy, probiotic yogurt, *Salvadora persica*, energy dispersive X-ray, polarized light microscope

Introduction

Dental caries is a dynamic process initiated by the demineralization of enamel, followed by the involvement of the deeper layers and resulting in cavitation.[1] White spot lesions, also known as surface-softened defects or incipient lesions, represent the initial stage of carious lesions. The lesions clinically manifest as opaque white areas when air-dried. Histologically, they exhibit an area of demineralization with an intact surface layer.[2] The presence of adequate amounts of calcium, phosphate and fluoride ions in saliva promotes the formation of fluorapatite, which enhances the process of remineralization. Unfortunately, in patients at high risk, the calcium and phosphate levels in saliva may not be sufficient for complete remineralization. Consequently, the supplementation of calcium and phosphate ions is essential for an effective remineralization process.[3]

Numerous non-invasive remineralization strategies were used in the clinical management of caries.[1] In recent years, several remineralizing agents have been developed and investigated. Milk and milk products play a unique role in remineralization due to their protective effects against the development of dental caries.[4] Additionally, the use of probiotics against bacteria-mediated diseases has recently been highlighted. The term "probiotic" was first introduced by Ferdinand Vergin in 1954 and originates from a Greek word meaning "for life".[5] According to the Food and Agriculture Organization/World Health Organization (FAO/WHO) (2001),[6] probiotics are defined as live bacteria that confer a benefit to the host when consumed in an appropriate amount. The harmless bacteria can compete with pathogens that threaten the host.[7] Most probiotics belonging to the *Lactobacillus* or *Bifidobacterium* genera have been shown to have a significant effect on the prevention of dental caries by inhibiting the growth properties of *Streptococcus mutans* bacteria.[8–10] Moreover, the concentration of calcium in the saliva is used as an indicator to estimate the balance between the demineralization and remineralization stages of enamel.[11] Many authors have asserted that the presence of a high concentration of calcium in yogurt can stabilize the oral biofilm and reduce the probability of dental caries formation.[7,9,12]

Miswak (*Salvadora persica*) is a natural product that has been widely used in many Muslim countries for the maintenance of oral health since ancient times. The product is also known by several other names, including the Arak tree, the mustard tree and the natural toothbrush tree. The miswak is a wooden stick that is used to clean the teeth and gums. It has been demonstrated to exert antibacterial, antifungal, antioxidant, and anticariogenic effects by elevating the pH of the plaque after the consumption of sucrose.[13]

The therapeutic and mechanical effects of miswak may be attributed to its constituents, such as calcium, phosphorus and fluoride, which can harden the outer surface of enamel and make it more resistant to caries. Similarly, trimethylamine, another constituent of miswak, can alter the bacterial contents of dental plaque due to its antibacterial effects.[14]

This study aimed to compare the remineralizing effect of *S. persica* and probiotic yogurt on artificially produced enamel lesions in order to identify the most effective natural product that can inhibit demineralization and promote the remineralization process in a carious lesion.

Material and methods

Sample size calculation

A sample size of 20 was calculated for each group, with a confidence interval (*CI*) of 95%, power of 80% and a significance level of 0.001.

Miswak extract

The roots of fresh miswak were chopped into tiny pieces and dried for 3 days at room temperature. Then, the pieces were ground using an electric grinder (Brightsail Industries Group Co., Ltd., Jiangyin, China). Subsequently, 10 g of the powder was added to 100 mL of ethanol in a sterile bottle and allowed to soak at 4°C for 48 h. Thereafter, centrifugation (Thermo IEC CL40R; Thermo Fisher Scientific, Waltham, USA) of all samples was conducted at 2,000 rpm for 15 min. The liquid content was then evaporated using a rotary evaporator (R-1010; Keda Machinery and Instrument Equipment Co., Ltd., Zhengzhou, China). The supernatant extract was collected and stored in the refrigerator in order to be used within 1 week.[15]

Preparation of casein phosphopeptide additives

A probiotic yogurt (including Bifidobacterium lactis BB-12 and *Lactobacillus acidophilus*) was used due to its high concentration of casein phosphopeptides (CPP). The yogurt (4,000 g) was centrifuged (Thermo IEC CL40R; Thermo Fisher Scientific) at 25°C for 10 min, resulting in the formation of 2 distinct portions: an insoluble portion precipitated at the base of the test tube; and a soluble portion (the supernatant containing CPP). The suspension was collected after 3 rounds of centrifugation and stored for further use.[16]

Tooth preparation

A total of 40 maxillary premolar teeth, extracted for orthodontic purposes, were collected from the outpatient dental clinic at the Faculty of Oral and Dental Medicine, Nahda University in Beni Suef, Egypt. The teeth were cleaned to remove debris and blood immediately after

extraction and sterilized using an autoclave (40 min at 120°C and 776 mmHg). Teeth exhibiting obvious signs of caries, restorations or cracks were excluded from the study. The selected teeth were soaked in 1 mL of sodium hydroxide (NaOH) for 48 h to remove any residual pellicle and kept in 0.1% thymol solution until further use. Each tooth was sectioned longitudinally into 2 identical halves in a buccolingual direction using a microtome (Leica SP 1600; Leica Microsystems, Wetzlar, Germany). Only the buccal halves were included in this study. Standardized windows (5 mm × 3 mm) were isolated on the buccal surface of enamel in each tooth using nail polish.

Grouping of teeth

The samples (N = 40) were divided into 4 groups, as follows: a negative control group (−ve CG) (n = 40), which was comprised of normal teeth before demineralization; a positive control group (+ve CG) (n = 40), which consisted of demineralized teeth without treatment; an experimental group I (EGI) (n = 20), which consisted of teeth remineralized with *S. persica*; and an experimental group II (EGII) (n = 20), which was comprised of teeth remineralized with probiotic yogurt.

Before demineralization, the samples were examined using scanning electron microscopy (SEM), energy dispersive X-ray (EDX) and polarized light microscopy. White spot lesions were induced by soaking the samples in a demineralizing solution (2.2 mM of $CaCl_2 \cdot 2H_2O$, 2.2 mM of KH_2PO_2 and 45 mM of acetate; pH = 4.6) and incubating them at 37°C for 96 h.[17] The demineralized samples were randomly divided into 2 experimental groups, according to the type of remineralizing solution used (*S. persica* extract or probiotic yogurt). The samples were soaked in the remineralizing solutions at 37°C for 8 days, and the solution was changed every 2 days. The treated samples were then immersed in distilled water until testing.

SEM

The buccal segments were anchored on stubs without coating and examined using SEM (JSM-IT200 InTouchScope™; JEOL USA, Inc., Peabody, USA) to evaluate the morphological changes on the tooth surface. The analysis was performed using the following parameters: an acceleration voltage of 25 kV, a working distance of 15 mm and pressure in the pulp chamber of 5–10 Pa.

Polarized light microscopic assessment

Longitudinal sections (200-µm thickness) of the samples were obtained using a microtome (Leica SP 1600; Leica Microsystems) and observed under a polarized light microscope (PriorLux POL™; PRIOR scientific, Cambridge, UK) using Canada balsam as the imbibition medium. Photomicrographs were taken at ×100 magnification and analyzed using the Image J software version v. 1.48q (https://imagej.net/ij/index.html).

EDX analysis

The quantitative mineral content of the surfaces was calculated using the EDX analysis. The amount of calcium and phosphorus (wt%) was calculated for all groups at baseline, after demineralization and after remineralization.

Statistical analysis

The IBM SPSS Statistics for Windows software v. 18 (SPSS Inc., Chicago, USA) was used to analyze the results. Data is presented as mean and standard deviation. The normality of the data was examined using the Kolmogorov–Smirnov and Shapiro–Wilk tests. One-way analysis of variance (ANOVA) was used to compare the tested elements in different groups, and pairwise differences were detected by Tukey's post hoc test. Two-sided p-values ≤0.005 were considered statistically significant.

Results

SEM observations in different groups

−ve CG

Scanning electron microscopy examination of the −ve CG samples revealed normal enamel surface morphology, enamel rod ends in some areas with horizontal lines at equal intervals (perikymata grooves), and ridges. Additionally, multiple fine scratches and scattered pores were observed on the enamel surface (Fig. 1A,B).

+ve CG

In the +ve CG, SEM examination after demineralization revealed a porous enamel surface (Fig. 1C). The prism cores exhibited erosion, with the prism peripheries remaining intact. Additionally, relatively narrow and shallow cracks were observed. Some areas demonstrated type I etching patterns with erosion of the rod cores and preservation of the prism peripheries. Areas of type II etching patterns were observed, with predominantly eroded interrod regions, leaving behind raised bumps representing the enamel rods (Fig. 1D).

EGI

An apparent reduction in the porosity of the enamel surface with a decrease in the number of type I etching patterns was observed in the EGI (Fig. 1E). Some rod holes were completely obliterated with globular precipitates, while others were empty (Fig. 1E,F).
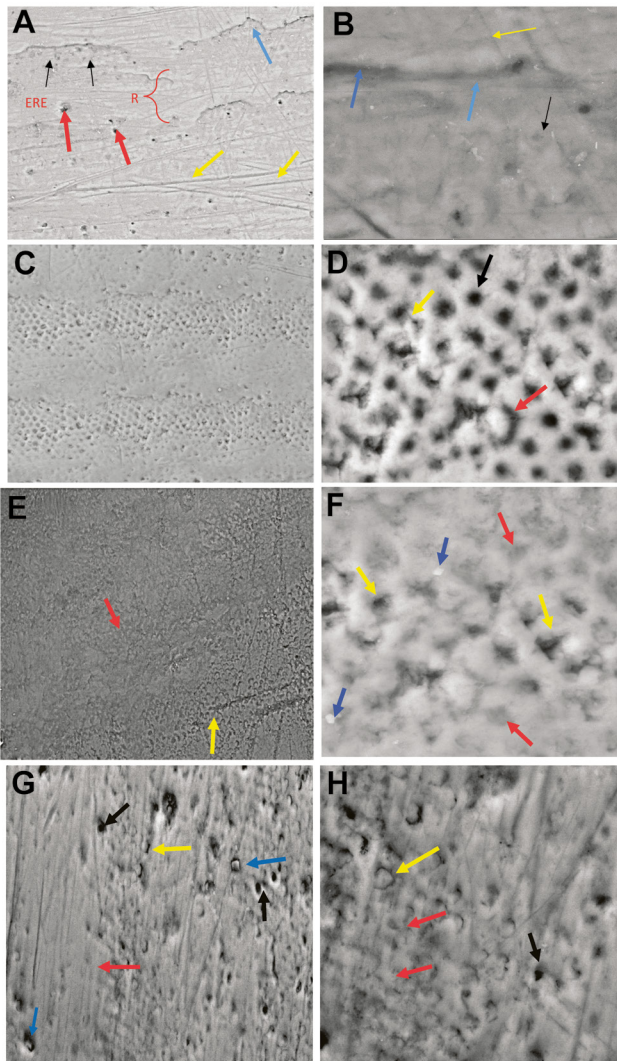
Fig. 1. Scanning electron micrographs of the enamel surfaces of specimens treated with miswak

A. Negative control group (−ve CG) sample showing enamel rod ends (ERE) in some areas (black arrows), perikymata grooves (blue arrow) and ridges (R). Multiple scratches (yellow arrows) and scattered pores (red arrows) were observed on the surface (×1000 magnification); B. Higher magnification of the previous image shows ERE (black arrow) and prikymata grooves (blue arrows). The surface contains a few scratches (yellow arrow) (×5000 magnification); C. Positive control group (+ve CG) sample showing a porous enamel surface (×1000 magnification); D. Higher magnification of the previous image shows the erosion of the prism cores with preserved prism peripheries (type I; black arrow), type II etching pattern with preferential loss of interrod enamel (red arrow), and narrow and shallow cracks (yellow arrow) (×5000 magnification); E. Experimental group I (EGI) sample showing a reduction in surface porosity, with completely obliterated prism cores (red arrow) and empty prism cores (yellow arrow) (×1000 magnification); F. Higher magnification of the previous image shows completely obliterated prism cores (red arrows), globular precipitation within the prism core (blue arrows) and empty prism cores (yellow arrows) (×5000 magnification); G. Experimental group II (EGII) sample showing completely obliterated prism cores (red arrow) and incompletely obliterated prism cores (yellow arrow). Globular precipitates were observed inside the prism cores (blue arrows), and pores were present in the samples (black arrows) (×1000 magnification); H. Higher magnification of the previous image shows completely obliterated prism cores (red arrows), a reduction in areas with type II etching pattern (yellow arrow), and enamel rod erosion as a result of demineralization (black arrow) (×5000 magnification).

EGII

In the EGII, prism cores completely obliterated with globular precipitates were observed on the enamel surfaces. Additionally, some incompletely obliterated prism cores were detected. The surface area of the type II etching pattern was reduced due to the thickening of the interrod enamel (Fig. 1G,H).

Polarized light microscopy observations in different groups

−ve CG

The samples in the −ve CG had a sound enamel surface and no signs of demineralization. An area of translucency was observed on the surface (Fig. 2A).

+ve CG

The samples in the +ve CG exhibited a relatively high degree of positive birefringence with the loss of the typical enamel structure within the lesion. This revealed a dark brown stain associated with the demineralization effect caused by the acid on the outer enamel surface (Fig. 2B).

EGI

The EGI presented with a birefringent zone, known as the remineralizing zone (RZ), on the surface of the treated
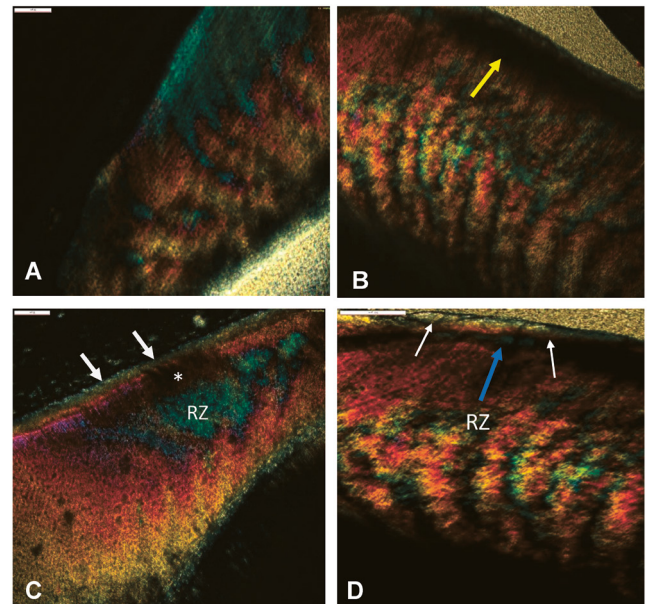
Fig. 2. Polarized light microscopy images of the samples (×40 magnification)

A. −ve CG sample showing an area of translucency with no signs of demineralization on the enamel surface; B. +ve CG sample showing a positive birefringence lesion area (yellow arrow); C. Remineralizing zone (RZ), an isolated area of demineralization (*) and a mineral precipitation band (white arrows) in the EGI sample; D. EGII sample showing the RZ (blue arrow) and a mineral precipitation band (white arrows).

enamel lesion. The zone was thick and more prominent, indicating a decrease in the body of the lesion. Additionally, mineral precipitation bands and isolated areas of demineralization were observed on the enamel surface (Fig. 2C).

EGII

In the EGII, a thin and less prominent negative birefringent zone was observed on the treated enamel surface, accompanied by the presence of a mineral precipitation band (Fig. 2D).

EDX calcium analysis

The −ve CG recorded the highest mean calcium value (49.1 ±1.85), followed by the EGI (48.1 ±1.28) and EGII (46.12 ±1.36), with the lowest value recorded in the

+ve CG (42.82 ±1.84). The results of the ANOVA revealed a statistically significant difference between the groups (p = 0.000). The mean value in the EGI was significantly different from those in the +ve CG and EGII, but no significant difference was observed between the −ve CG and EGI, as determined by Tukey's test (Table 1) (Fig. 3).

EDX phosphorus analysis

The −ve CG and EGI recorded the highest mean phosphorus values (20.26 ±0.45), followed by the EGII (20.00 ±0.74), with the lowest value recorded in the +ve CG (18.65 ±0.43). A statistically significant difference between the groups was observed using ANOVA (p = 0.000). The mean value in the +ve CG was found to be significantly different from those observed in the other groups. However, no significant differences were identified between the −ve CG, EGI and EGII (Table 1) (Fig. 3,4).

Table 1. Descriptive statistics and significant differences in calcium (Ca) and phosphorus (P) levels between the groups (analysis of variance (ANOVA) test)

Group		M	SD	SE	95% CI for M		Min	Max	F	p-value
					lower bound	upper bound				
Ca	−ve CG	49.10[a]	1.85	0.58	47.78	50.42	46.59	50.98	29.86	0.000*
	+ve CG	42.82[c]	1.84	0.58	41.5	44.13	40.36	44.78		
	EGI	48.10[a]	1.28	0.40	47.18	49.01	46.59	49.72		
	EGII	46.12[b]	1.36	0.43	45.15	47.09	44.78	47.98		
P	−ve CG	20.26[a]	0.45	0.14	19.94	20.59	19.88	20.9	20.94	0.000*
	+ve CG	18.65[b]	0.43	0.14	18.34	18.96	18.07	19.09		
	EGI	20.26[a]	0.45	0.14	19.94	20.59	19.88	20.9		
	EGII	20.00[a]	0.74	0.23	19.47	20.53	19.09	20.9		

Data presented as wt%. M – mean; SD – standard deviation; SE – standard error; CI – confidence interval; −ve CG – negative control group; +ve CG – positive control group; EGI – experimental group I; EGII – experimental group II; * statistically significant ($p \leq 0.005$, Tukey's post hoc test). Different superscript letters indicate statistically significant differences between the groups.
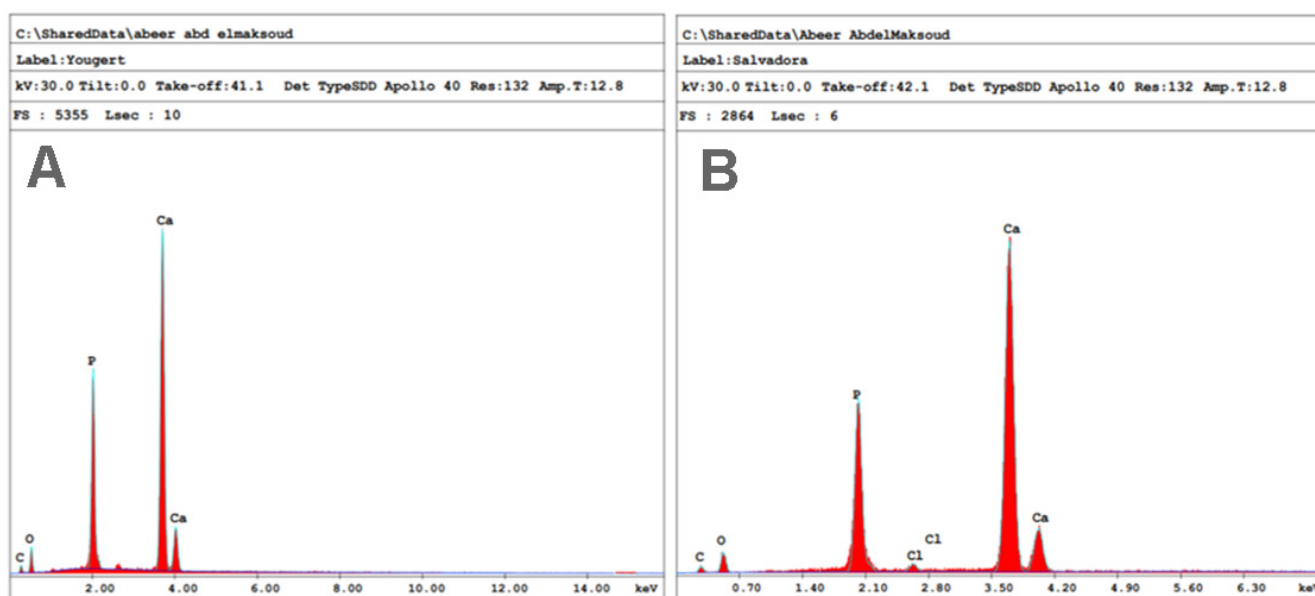
Fig. 3. Energy dispersive X-ray (EDX) element analysis in different groups
A. Probiotic yogurt; B. *Salvadora persica*.
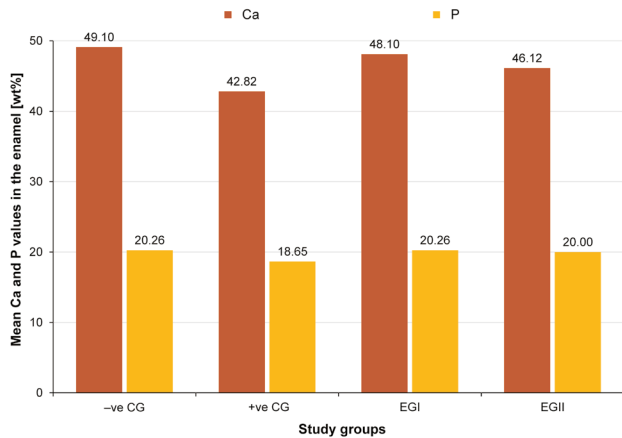
Fig. 4. Mean calcium (Ca) and phosphorus (P) values in the enamel in different groups

Discussion

Initial caries pass through different stages, starting from changes in the molecular structure of the tooth's hydroxyapatite crystal and ending with the formation of white spot lesions. The progression of these stages is enhanced by a continuous imbalance between protective and pathological variables, which may result in the dissolution of the hydroxyapatite crystals and the initiation of the demineralization process.[18]

Modern dentistry has focused on the treatment of initial caries (non-cavitated or white spot lesions) and the replenishment of minerals lost during remineralization. This process helps to precipitate minerals on the demineralized enamel surface, thereby reducing the progression of the disease and enhancing the aesthetics.[19]

This study aimed to evaluate the effectiveness of miswak in remineralizing initial carious lesions in enamel compared to that of probiotics. The SEM and polarized light microscopic results indicate a significant increase in the level of remineralization in all the tested samples treated with miswak. This finding is consistent with another study, which reported an increase in enamel hardness after brushing with miswak.[20] Fluoride has a high affinity for binding with OH ions in hydroxyapatite, forming fluorapatite, which replaces the demineralized hydroxyapatite.[21] In another study, different concentrations (5% and 10%) of miswak were applied to demineralized enamel surfaces for 7 days, and a remineralizing effect was observed, attributed to the presence of fluoride in the extract.[22]

Another potential explanation for the defensive effects of miswak versus citric acid is the immediate formation of a polymer on the enamel surface. This polymer is reported to have a protective effect against further demineralization.[15] One study evaluated the effect of fluoridated miswak sticks on demineralized lesions that appeared after removing an orthodontic appliance and reported enhanced remineralizing effects on the demineralized areas.[23]

The antibacterial effect of miswak is attributed to the presence of many active compounds such as trimethylamine, fluoride, vitamin C, salvadorine chlorides, silica, sulfur, and small amounts of saponins, flavonoids, sterols, and tannins. Studies have demonstrated that *S. persica* inhibits the growth and colonization of *S. mutans*.[13,24] Al-Sohaibani and Murugan showed that the antibacterial effect of *S. persica*, which is extracted from the bark, pulp, or both, may be due to the presence of trimethylamine, sodium chloride, potassium chloride, and salvadourea.[24] Darout et al. correlated the antimicrobial effect of miswak with the presence of anionic components, including thiocyanate.[25] The leaching of thiocyanate from *S. persica* has been demonstrated to elevate salivary thiocyanate levels and improve the effectiveness of the hydrogen peroxide–peroxidase–thiocyanate system in the saliva.[25,26] Another study has shown that the aqueous extraction of miswak released more ionic concentrations of calcium, phosphorus and fluoride than the alcoholic extract, which may account for its superior remineralization effect.[27] To the best of our knowledge, there is no evidence that miswak has an allergenic effect. However, 1 case report published in 2017 reported the occurrence of allergic contact stomatitis after the use of *S. persica* toothpaste. The author concluded that it is a very rare condition.[28]

The probiotic yogurt used in this study was free from any artificial sugars, in order to avoid their cariogenic effects. Even though yogurt contains the intrinsic lactose sugar (cariostatic), it was proven to be the least cariogenic sugar of all mono- and disaccharides. Milk yogurts may have a protective effect on the teeth, but only when no sugars are added to them.[29] However, the results of the current study indicated that the probiotic yogurt extract may also have a protective effect against caries. This finding is in accordance with those of previous studies, which correlated a decrease in dental caries with daily consumption of dairy products.[16,30–32] Further analysis of the various constituents of dairy products revealed that each constituent plays a unique role in enhancing the remineralization effect. The protein and fat content in milk may inhibit the demineralization effect of acidogenic bacteria and promote the formation of a protective barrier against further mineral loss.[16] On the other hand, the enzymes present in milk may play a key role in diminishing the growth of cariogenic bacteria.[30–32]

The protective action of yogurt against dental caries may be attributed to the similarity between the components in yogurt and milk. Furthermore, yogurt has additional properties that enhance its remineralization effect. The high protein concentration in yogurt results in the incorporation of dry milk with low or no fat during processing. Moreover, the concentrations of calcium and phosphorus in yogurt are the same as those in milk. However, calcium remains in its ionic form due to the characteristic low pH of yogurt. Furthermore, yogurt contains a higher concentration of CPP than milk. These differences enhance the remineralization effects of yogurt compared to dairy milk.[33]

The EDX analysis revealed that the mineral content of the 2 remineralizing solutions differed significantly. In particular, the concentrations of calcium and phosphorus in *S. persica* were found to be significantly higher than those in yogurt. Furthermore, the application of *S. persica* on demineralized surfaces can elevate calcium and phosphorus levels to their original values. These findings indicate that miswak is a suitable remineralizing agent for the treatment of white spot lesions. Further studies are necessary to assess the remineralization effects of various casein derivatives and natural products on the enamel surface. Similar experiments should be conducted in vivo (on animals) to evaluate the action of these elements on surrounding vital structures. Further studies are required on both experimental groups (*S. persica* and yogurt) using different methodologies.

Conclusions

In the present study, *S. persica* effectively restored the minerals that had been lost during the demineralization of enamel. The effects of *S. persica* were superior to those of probiotic yogurt.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Research Ethics Committee of the Faculty of Oral and Dental Medicine at Nahda University in Beni Suef, Egypt (approval No. 010722). Written informed consent was obtained from the study participants prior to their inclusion in the study.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Noura Mohammed Bakr https://orcid.org/0000-0002-5907-6689
Abeer Abdelmaksoud Mohamed https://orcid.org/0000-0002-2619-7548
Ghada Abdelwahab Salem https://orcid.org/0000-0001-9217-526X

References

1. Huysmans MCDNJM, Chew HP, Ellwood RP. Clinical studies of dental erosion and erosive wear. *Caries Res*. 2011;45 Suppl 1:60–68. doi:10.1159/000325947
2. Khoroushi M, Kachuie M. Prevention and treatment of white spot lesions in orthodontic patients. *Contemp Clin Dent*. 2017;8(1):11–19. doi:10.4103/ccd.ccd_216_17
3. Ekambaram M, Mohd Said SNB, Yiu CKY. A review of enamel remineralisation potential of calcium- and phosphate-based remineralisation systems. *Oral Health Prev Dent*. 2017;15(5):415–420. doi:10.3290/j.ohpd.a38779
4. Thimmaiah C, Shetty P, Shetty SB, Natarajan S, Thomas NA. Comparative analysis of the remineralization potential of CPP-ACP with fluoride, tri-calcium phosphate and nano hydroxyapatite using SEM/EDX – An in vitro study. *J Clin Exp Dent*. 2019;11(12):e1120–e1126. doi:10.4317/jced.55941
5. Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients*. 2017;9(9):1021. doi:10.3390/nu9091021
6. Mack DR. Probiotics-mixed messages. *Can Fam Physician*. 2005;51(11):1455–1457,1462–1264. PMID:16353824.
7. Bustamante MF, Agustín-Perez M, Cedola F, et al. Design of an anti-inflammatory diet (ITIS diet) for patients with rheumatoid arthritis. *Contemp Clin Trials Commun*. 2020;17:100524. doi:10.1016/j.conctc.2020.100524
8. Wasfi R, Abd El-Rahman OA, Zafer MM, Ashour HM. Probiotic *Lactobacillus* sp. inhibit growth, biofilm formation and gene expression of caries-inducing *Streptococcus mutans*. *J Cell Mol Med*. 2018;22(3):1972–1983. doi:10.1111/jcmm.13496
9. Vuotto C, Longo F, Donelli G. Probiotics to counteract biofilm-associated infections: Promising and conflicting data. *Int J Oral Sci*. 2014;6(4):189–194. doi:10.1038/ijos.2014.52
10. Lee Y, Yoon Y, Choi KH. Probiotics-mediated bioconversion and periodontitis. *Food Sci Anim Resour*. 2021;41(6):905–922. doi:10.5851/kosfa.2021.e57
11. Farooq I, Bugshan A. The role of salivary contents and modern technologies in the remineralization of dental enamel: A narrative review. *F1000Res*. 2020;9:171. doi:10.12688/f1000research.22499.3
12. Malavalli PL, Shetty SB, Thimmaiah C, Ramlan A, Hugar SM, Meharwade P. Evaluation of the effect of probiotic yogurt consumption on salivary pH, buffering capacity and calcium level in 6–12-year-old children: An in vivo study. *Int J Clin Pediatr Dent*. 2022;15(2):194–198. doi:10.5005/jp-journals-10005-2368
13. Haque MM, Alsareii SA. A review of the therapeutic effects of using miswak (*Salvadora persica*) on oral health. *Saudi Med J*. 2015;36(5):530–543. doi:10.15537/smj.2015.5.10785
14. Khunkar S, Hariri I, Alsayed E, et al. Inhibitory effect of *Salvadora persica* extract (miswak) on collagen degradation in demineralized dentin: In vitro study. *J Dent Sci*. 2021;16(1):208–213. doi:10.1016/j.jds.2020.05.025
15. Bawazeer TM, Alsoufi MS, Katowah D, Alharbi WS. Effect of aqueous extracts of *Salvadora persica* "miswak" on the acid eroded enamel surface at nano-mechanical scale. *MSA*. 2016;7(11):754–771. doi:10.4236/msa.2016.711059
16. Ferrazzano GF, Cantile T, Quarto M, Ingenito A, Chianese L, Addeo F. Protective effect of yogurt extract on dental enamel demineralization in vitro. *Austr Dent J*. 2008;53(4):314–319. doi:10.1111/j.1834-7819.2008.00072.x
17. Nobahar S, Mirzaeei S, Simaei L, Ahmadi Z. Comparative study of the effect of flavonoids and the whey extract on enamel micro-hardness: An in vitro study. *Avicenna J Dent Res*. 2020;12(3):81–85. doi:10.34172/ajdr.2020.17
18. Geeta RD, Vallabhaneni S, Fatima K. Comparative evaluation of remineralization potential of nanohydroxyapatite crystals, bioactive glass, casein phosphopeptide-amorphous calcium phosphate, and fluoride on initial enamel lesion (scanning electron microscope analysis) – An in vitro study. *J Conserv Dent*. 2020;23(3):275–279. doi:10.4103/JCD.JCD_62_20
19. Philip N. State of the art enamel remineralization systems: The next frontier in caries management. *Caries Res*. 2019;53(3):284–295. doi:10.1159/000493031
20. Dianti F, Triaminingsih S, Irawan B. Effects of miswak and nano calcium carbonate toothpastes on the hardness of demineralized human tooth surfaces. *J Phys: Conf Ser*. 2018;1073(3):032008. doi:10.1088/1742-6596/1073/3/032008
21. Pajor K, Pajchel L, Kolmas J. Hydroxyapatite and fluorapatite in conservative dentistry and oral implantology – A review. *Materials (Basel)*. 2019;12(17):2683. doi:10.3390/ma12172683

22. Hoobi NM, Hussein B, Qasim AA, Abdulrahman M. Dissolution of calcium ion from teeth treated with different concentrations of siwak water extract in comparison with sodium fluoride. *J Bagh Coll Dent*. 2014;26(1):166–170. https://www.semanticscholar.org/paper/Dissolution-of-calcium-ion-from-teeth-treated-with-Hoobi-Hussein/e817b48a8f51a5d2ce6c6abf9f5449c7936b9800. Accessed June 10, 2024.

23. Baeshen HA, Lingström P, Birkhed D. Effect of fluoridated chewing sticks (miswaks) on white spot lesions in postorthodontic patients. *Am J Orthod Dentofacial Orthop*. 2011;140(3):291–297. doi:10.1016/j.ajodo.2010.04.034

24. Al-Sohaibani S, Murugan K. Anti-biofilm activity of *Salvadora persica* on cariogenic isolates of *Streptococcus mutans*: In vitro and molecular docking studies. *Biofouling*. 2012;28(1):29–38. doi:10.1080/08927014.2011.647308

25. Darout IA, Christy A, Skaug N, Egeberg PK. Identification and quantification of some potentially antimicrobial anionic components in miswak extract. *Indian J Pharmacol*. 2000;32(1):11–14. https://www.researchgate.net/publication/286955247. Accessed June 10, 2024.

26. Bhat PK, Kumar A, Sarkar S. Assessment of immediate antimicrobial effect of miswak extract and toothbrush on cariogenic bacteria – A clinical study. *J Adv Oral Res*. 2012;3(1):25–29. doi:10.1177/2229411220120106

27. Wassel MO, Sherief DI. Ion release and enamel remineralizing potential of miswak, propolis and chitosan nano-particles based dental varnishes. *Pediatr Dent J*. 2019;29(1):1–10. doi:10.1016/J.PDJ.2018.12.004

28. Chaubal TV, Bapat RA, Bapat PR. Allergic contact stomatitis caused *Salvadora persica* in toothpaste. *Contact Dermatitis*. 2017;77(5):325–326. doi:10.1111/cod.12819

29. Varghese L, Varughese JM, Varghese NO. Inhibitory effect of yogurt extract on dental enamel demineralisation – An in vitro study. *Oral Health Prev Dent*. 2013;11(4):369–374. doi:10.3290/j.ohpd.a30604

30. Levine RS. Milk, flavoured milk products and caries. *Br Dent J*. 2001;191(1):20. doi:10.1038/sj.bdj.4801080

31. Merritt J, Qi F, Shi W. Milk helps build strong teeth and promotes oral health. *J Calif Dent Assoc*. 2006;34(5):361–366. PMID:16900979.

32. Wang J, Jin G, Gu K, Sun J, Zhang R, Jiang X. Association between milk and dairy product intake and the risk of dental caries in children and adolescents: NHANES 2011–2016. *Asia Pac J Clin Nutr*. 2021;30(2):283–290. doi:10.6133/apjcn.202106_30(2).0013

33. Sfakianakis P, Tzia C. Conventional and innovative processing of milk for yogurt manufacture; Development of texture and flavor: A review. *Foods*. 2014;3(1):176–193. doi:10.3390/foods3010176

Antibacterial and preventive effects of newly developed modified nano-chitosan/glass-ionomer restoration on simulated initial enamel caries lesions: An in vitro study

Heba Abdelhamid Shalaby[1,2,A–F], Nofal Khamis Soliman[3,B–D], Khaled Wagih Al-Saudi[4,C–F]

[1] Department of Dental Biomaterials, Faculty of Oral and Dental Medicine, Nahda University, Beni Suef, Egypt

[2] Department of Dental Biomaterial, Faculty of Dentistry, Fayoum University, Faiyum, Egypt

[3] Department of Basic Sciences, Nahda University, Beni Suef, Egypt

[4] Department of Operative and Conservative Dentistry, Faculty of Oral and Dental Medicine, Nahda University, Beni Suef, Egypt

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

Abstract

Background. Despite the superiority of glass-ionomer cements (GICs) over composites in treating white spot lesions (WSLs), there is still a concern about their preventive and antibacterial properties. Efforts have been made to improve the strength of their bond to demineralized enamel, fluoride release and antibacterial properties by adding nanoparticles of chitosan, which seems to be a promising method.

Objectives. The aim of the present study was to assess the antibacterial effect, the microshear bond strength (μSBS) to enamel at the WSL area, and the fluoride and nano-chitosan release after modifying the polyacrylic acid liquid phase of a traditional GIC with different nano-chitosan volumes.

Material and methods. A total of 120 samples were prepared, and then divided into 4 groups ($n = 30$): G1 – non-modified GIC, which served as a control group, while G2, G3 and G4 were modified with different nano-chitosan volumes (50%, 100% and 150%, respectively). Microshear bond strength was assessed using a universal testing machine (UTM) after storage in distilled water for 24 h. Fluoride and nano-chitosan release was measured with the use of spectrophotometers at different time points (initially, and at 1 h, 24 h, 48 h, 72 h, 1 week, 2 weeks, 3 weeks, and 6 weeks) after storage in distilled water. The antibacterial effect against the Streptococcus aureus strain was assessed with the agar diffusion test. The data was statistically analyzed.

Results. After 24-hour storage, G2 recorded a slight, yet non-significant, increase in the μSBS values (4.1 ±0.94 MPa) as compared to G1 (3.9 ±1.30 MPa). With regard to fluoride release, the amount recorded for G1 was significantly greater at the end of the 24-hour storage period (0.70 ±0.30 μmf/cm^2) than modified nano-chitosan GIC groups; G1 was followed by G4 (0.54 ±0.34 μmf/cm^2). The highest amount of nano-chitosan release after 24-hour storage was noted for G3 (0.85 ±0.00 μmf/cm^2). The highest inhibition zone value was recorded for G2.

Conclusions. Glass-ionomer cement modified with 50% nano-chitosan was shown to positively affect μSBS and the antibacterial effect, while modification with 150% nano-chitosan significantly increased fluoride release.

Keywords: preventive, glass-ionomer, nano-chitosan, antibacterial, initial caries

Introduction

White spot lesions (WSLs) are areas of non-cavitated sub-surface demineralization, which are considered initial caries. Initial enamel caries is due to bacterial activity that leads to demineralization and causes micro-porosities between enamel rods. The refractive index is altered and enamel translucency decreases, showing chalky white marks on enamel surface, with darker patches.[1] White spot lesions occur as a result of fixed orthodontic treatment. Thus, it is mandatory for a treatment protocol for these patients to have additional steps to manage WSLs during and at the end of the treatment.[2]

Many approaches have been used to manage WSLs, such as topical fluoride application, in which the structure of the tooth is altered due to fluoroapatite, thus becoming more resistant to caries. Another approach consists in compensating for the loss of minerals by utilizing fillings capable of mineral release. Other approaches manage the already defected part by utilizing composite restoration, or combining the effects of restoration and preventing further decay activity by utilizing glass-ionomer restorations.[3]

Many investigations conducted over the last 2 decades have shown that glass-ionomer cements (GICs) are superior to resin composites in managing the decalcification of WSLs due to their anti-cariogenic activity through fluoride release.[4] However, GICs have some drawbacks related to their greater sensitivity to water contamination, brittleness and poorer mechanical characteristics as compared to resin composites.[1]

To overcome some of these limitations, GICs have undergone significant alteration through incorporating various additives, such as zirconia, glass fibers, hydroxyapatite, bioactive glass particles, and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).[5] Glass-ionomer cements have antibacterial qualities owing to their low pH during the setting reaction as well as fluoride release by ion leaching, lowering demineralization, enhancing remineralization, and preventing the development of pellicles and biofilm, and bacterial metabolism and growth. However, the antibacterial efficacy of traditional GICs is still insufficient with regard to the adhesion, cell viability and biofilm formation of *Streptococcus mutans*.[6] As a result, clinical advantages may be obtained by combining antibacterial additives or modifiers with traditional GICs without sacrificing their mechanical and physical properties, fluoride release, and adhesion to hard tooth structures.[7]

Chitosan is a naturally occurring linear biopolyaminosaccharide produced by alkaline deacetylation of chitin found in crab and shrimp shells. It is widely used as a biopolymer. Chitosan is a weak base that is insoluble in water and organic solvents, but it may be dissolved in diluted aqueous acidic solutions like acetic acid. It is also characterized by biodegradability, biocompatibility, muco-adhesion, and a broad range of antibacterial and antibiofilm capabilities against both Gram-positive and Gram-negative microorganisms.[8,9] Meanwhile, pharmaceutical companies frequently employ nanosized chitosan formulations as medication delivery systems. It has been suggested that nanoscale chitosan (nano-chitosan) particles may have an advantage over larger chitosan particles due to their increased surface area and charge density, which may improve their interaction with the medium (i.e., tooth surface).[10]

Therefore, the present study aimed to investigate the effect of modifying the polyacrylic acid (PAA) liquid phase of a traditional GIC with different nano-chitosan volume content on microshear bond strength (µSBS) to enamel at the WSL area, fluoride and nano-chitosan release, and antibacterial properties in comparison with a traditional GIC. The null hypotheses were that the highest nano-chitosan volume GIC would be superior in all of the following: (1) µSBS of GIC to the enamel lesion; (2) fluoride and nano-chitosan release; and (3) antibacterial properties.

Material and methods

Nano-chitosan was prepared from chitosan (medium-molecular-weight deacetylated chitin, poly-D-glucosamine 75–80% deacetylation; Sigma-Aldrich, St. Louis, USA). First, a 1% aqueous acetic acid solution was prepared. Then, 0.7 mg of nano-chitosan powder was magnetically stirred in 100 mL of the aqueous acetic acid solution for 24 h at room temperature. The pH value was adjusted to 3.7 with the Tris hydroxymethyl amino methane HCl buffer. The nano-chitosan solution was properly sealed and stored in a refrigerator until needed for addition to the GIC (Promedica Dental Material, Neumünster, Germany).[8,10]

Study design

A total of 120 samples were prepared, of which 80 samples were disk specimens for material preparation, and the remaining 40 samples were human permanent premolar teeth. The samples were divided into 4 equal groups ($n = 30$), taking into account the amount of GIC powder divided by the PAA liquid to nano-chitosan ratio of the utilized GIC. According to the manufacturer's instructions, 0.12 mg of powder was mixed with 0.5 mL of PAA liquid (1/1 V) to prepare the control GIC (CGIC) disks (the control group G1). The cement was manually mixed using a spatula on a paper pad. One volume of PAA liquid was substituted by adding different chitosan solutions (0.125 mL, 0.250 mL and 0.375 mL) and was mixed with 1 volume of CGIC powder to form different investigated groups:

G2: 1/(1:~0.5 V); G3: 1/(1:1 V); and G4: 1/(1:1.5 V), i.e., nearly 50%, 100% and 150% by volume nano-chitosan-modified GIC for groups G2, G3 and G4, respectively. Group G1 (conventional glass-ionomer powder/PAA liquid (1/1 V)) served as a control group,

Each group was then further subdivided into 3 equal subgroups according to the test utilized (n = 10): SG1 – µSBS test; SG2 – fluoride and nano-chitosan release; and SG3 – bacterial culture.

Selection of the teeth

A total number of 40 caries-free human permanent premolar teeth extracted for orthodontic purposes were utilized.[11] The teeth were carefully checked, then rinsed with water and scaled with a periodontal scaler to remove any blood, attached periodontal tissues, plaque, and calculus. To avoid microbial growth, they were kept in an incubator with distilled water and 0.5% thymol.[12]

Production of artificial enamel white spot lesions

Teflon rings (15 mm in height and an internal diameter of 38 mm) were utilized to mount the selected teeth vertically in acrylic resin blocks.[13] An enamel window (3 × 3 mm) was scratched in the middle of cervical one-third on the buccal surface of the tooth, utilizing a diamond stone size 12 with gentle pressure and abundant irrigation. To achieve a consistent standard surface, enamel surfaces were hand-polished in a clockwise circular motion, using silicon carbide paper (grit 240, 400 and 600) for 30 s each under copious water coolant. Before applying the cement, the scratched enamel surfaces were treated with PAA for 30 s.[14]

Microshear bond strength test

Specimen fabrication

A transparent polyethylene tube obtained from a scalp vein infusion set (23 G; JMS Singapore, Singapore) was cut into small tubes ~0.75–1-millimeter-long, using a sharp lancet. The transparent polyethylene tubes (an external diameter of 2.35 mm and an internal diameter of 0.93 mm) were utilized to help in loading GIC and different modified nano-chitosan sample types with a small condenser (Primadent, Germany) in slight excess from the side to be bonded to tooth enamel.[15]

After the cement was fully hardened, the polyethylene tubes were carefully removed by lancet No. 11, leaving the cement micro-cylinders bonded to enamel surfaces. Then, the samples were checked using a magnifying glass ×6 for any defects or air bubbles. The bonded specimens were kept in an incubator with distilled water for 1 day.[16]

Specimen testing

After 24 h of storage, 4 tightening bolts were utilized to secure each specimen with its bonded GIC micro-cylinder to the lower half of a specifically designed attachment. The universal testing machine (UTM) had lower fixed and upper movable compartments (model LRX Plus; Lloyd Instruments Ltd., Fareham, UK), which were utilized to adjust the attachment jig with screws, with a load cell of 5 kN. The bonded GIC micro-cylinders were secured tight at their bases by utilizing a wire loop made from orthodontic stainless steel ligature wire (180 µm in diameter).[17]

Microshear load was applied by the tensile mode of force, utilizing the testing equipment with a crosshead speed of 0.5 mm/min (Fig. 1). Computer software was used to record the data (NEXYGEN™ MT; Lloyd Instruments Ltd.).[18] Microshear bond strength was then calculated in megapascals by dividing the load at failure [N] by the bonding area [mm²].[17] The calculated results were tabulated and statistically analyzed.

Fig. 1. Microshear load applied to the teeth by the tensile mode of force via a universal testing machine (UTM), utilizing a wire loop

Determination of fluoride and nano-chitosan release

Sample preparation

Cement disks (10 mm in diameter, 1.5 mm in thickness) were prepared utilizing a Teflon mold at room temperature (23 ±1°C) and controlled relative humidity (50 ±5%) according to ISO specification #9917-1:2007.[19] After the infusion of the cement into the mold, nylon thread was introduced into each cement disk during packing to allow suspension in the test medium.[20]

The surface of the cement materials was covered with Mylar strips and held between 2 glass slabs under constant finger pressure to expel excess cement, and allowed to set inside the mold for 20 min through chemical curing. Then, they were removed from the molds.[6]

Storage medium

Each disk was suspended vertically by nylon thread, and then immersed in a polyethylene vial containing 10 mL of deionized water for 6 weeks. Measurement readings were taken at different time intervals: initially, and at 1 h, 24 h, 48 h, 72 h, 1 week, 2 weeks, 3 weeks, and 6 weeks.[21]

Sample measurements

Fluoride release measurement

To determine the amount of fluoride release, a spectrophotometer with a fluoride ion-specific electrode (2007 Edition 2; Hach Company, Berlin, Germany) was connected to an ion digital analyzer for fluoride release reading at different time intervals. The amount of fluoride release was then calculated according to the following formula (Equation 1)[22]:

$$\text{amount of fluoride release} = \frac{\text{amount measured with the spectrophotometer}}{\text{surface area of the disk}} [\mu mf/cm^2] \quad (1)$$

Nano-chitosan release measurement

To assess the amount of nano-chitosan release at the different time intervals, an ultraviolet (UV) double-beam spectrophotometer (LAMBDA™; PerkinElmer, Boston, USA) was utilized. The maximum absorbance color was recorded at 254 nm at wavelengths of 190–800 nm.[23,24] The concentration of nano-chitosan released in distilled water was calculated using the Beer–Lambert law (Equation 2)[25]:

$$A = \varepsilon \times b \times c \ [A.U.] \quad (2)$$

where:
A – absorbance [A.U.];
ε – molar absorptivity [L·mol^{-1}·cm^{-1}];
b – path length of the beam in the absorbing medium [cm]; and
c – concentration of the absorbing species [M].

Bacterial culture test

Using the agar diffusion technique, disks from each cement mixture group were subjected to bacterial testing for *Staphylococcus aureus* (ATCC 25922), which was cultured on Muller–Hinton Broth, and then incubated at 37°C for 18 h. All cement mixture disks were sterilized with UV before the subsequent procedure.[26]

Bacterial suspension was vigorously agitated with a vortex mixer just before use. Then, saline was added gradually with matching turbidity, equivalent to a 0.5 McFarland standard.[27] The Muller–Hinton agar in the amount of 30 mL was spread to a thickness of 2 mm in Petri dishes (10 cm in diameter). Muller–Hinton Broth containing *S. aureus* was cultured over the Muller–Hinton agar.[28]

Four standard GIC mixture disks were placed within the agar, using fine-pointed sterile forceps. The disks were pressed firmly to ensure complete contact with the agar. After the incubation of the plates at 37°C for 24h, the degree of sensitivity was determined by measuring the easily visible and clear zone of inhibition produced by the diffusion of the antimicrobials from the disks into the surrounding medium. Finally, the sizes of inhibition zones were calculated by subtracting the diameter of the specimen from the average of the 5 halo measurements.[26]

Statistical analysis

All the collected data was statistically analyzed. The data is presented as mean and standard deviation (*M ±SD*). The repeated measures analysis of variance (ANOVA) was used to compare between the different tested groups and follow-up periods for all parameters, followed by pairwise comparison with the Bonferroni correction. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, v. 25.0 (IBM Corp., Armonk, USA).

Results

Microshear bond strength

The results of the μSBS test for different investigated groups are presented in Table 1 and Fig. 2. After 24 h of storage, the enamel structure of G2 showed a slight, yet non-significant, increase in the μSBS values (4.1 ±0.9 MPa) as compared to G1 (3.9 ±1.3 MPa), whereas G3 and

Table 1. Descriptive statistics and the test of significance for microshear bond strength (µSBS) [MPa] among different investigated groups after 24-hour storage

Groups (n = 10)	µSBS [MPa]	p-value
G1	3.9 ±1.30[a]	
G2	4.1 ±0.94[a]	0.020*
G3	2.5 ±0.62[b]	
G4	2.2 ±0.62[b]	

Data presented as mean ± standard deviation (*M ±SD*).
Groups: G1 (control) – non-modified glass-ionomer cement (GIC); G2 – GIC modified with 50% vol. chitosan; G3 – GIC modified with 100% vol. chitosan; and G4 – GIC modified with 150% vol. chitosan.
* statistically significant; different letters in superscript denote statistically significant differences (paired samples *t* test).
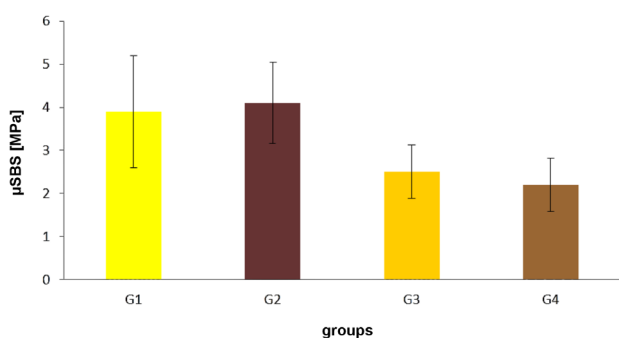
Fig. 2. Histogram showing microshear bond strength (µSBS) [MPa] among different investigated groups after 24-hour storage

G4 recorded significantly smaller µSBS of their enamel structure (2.5 ±0.62 MPa and 2.2 ±0.62 MPa, respectively). However, statistical analysis revealed no significant differences between both groups.

Rate of fluoride release

The results of the rate of fluoride release after immersion and storage in a distilled water solution at different time intervals (initially, and at 1 h, 24 h, 48 h, 72 h, 1 week, 2 weeks, 3 weeks, and 6 weeks) for different investigated groups are presented in Table 2.

Group G1 recorded a significantly greater rate of fluoride release initially (1.70 ±0.21 µmf/cm^2) and at the end of the 24-hour storage period (0.70 ±0.30 µmf/cm^2) than the modified nano-chitosan GIC groups. Among the latter, G4 recorded the greatest amount of fluoride release after 24 h (0.54 ±0.34 µmf/cm^2), followed by G3 (0.40 ±0.90 µmf/cm^2); G2 recorded the lowest fluoride release (0.30 ±0.20 µmf/cm^2).

The minimum rate of fluoride release was recorded in the period between 48 h and 1 week from immersion for all the investigated groups.

The maximum rate of fluoride release was recorded after 2 weeks from immersion for G1, G2, G3, and G4 at 4.31 ±0.74 µmf/cm^2, 4.13 ±0.75 µmf/cm^2, 4.37 ±0.76 µmf/cm^2, and 4.15 ±0.68 µmf/cm^2. Changes in the fluoride release rate for the samples immersed for 2 weeks as compared to 3 weeks were non-significant.

Rate of nano-chitosan release

The results of the rate of nano-chitosan release after immersion and storage in a distilled water solution at different time intervals (initially, and at 1 h, 24 h, 48 h, 72 h, 1 week, 2 weeks, 3 weeks, and 6 weeks) for different investigated groups are presented in Table 3.

The highest amount of nano-CS release was recorded for G3 – initially (1.09 ±0.00 µmf/cm^2) and after 24 h (0.85 ±0.00 µmf/cm^2). Groups G2 and G4 recorded the lowest amount of nano-chitosan release; they also showed the same values – initially (0.53 ±0.01 µmf/cm^2) and after 24 h (0.60 ±0.00 µmf/cm^2).

After 1 week, the rate of nano-chitosan release was constant for all the investigated groups (0.30 ±0.00 µmf/cm^2, 0.30 ±0.00 µmf/cm^2 and 0.78 ±0.00 µmf/cm^2 for G1, G2 and G3, respectively).

Table 2. Descriptive statistics and the test of significance for the rate of fluoride release [µmf/cm^2] for the tested groups at different investigated time intervals

Time point	Rate of fluoride release [µmf/cm²]				p-value
	G1	G2	G3	G4	
Initially	1.70[a] ±0.21	1.42[c] ±0.25	1.69[b] ±0.13	1.66[b] ±0.28	0.807
1 h	0.55[a] ±0.07	0.29[b] ±0.07	0.55[c] ±0.07	0.54[c] ±0.27	0.002*
24 h	0.70[a] ±0.30	0.30[d] ±0.20	0.40[cd] ±0.90	0.54[b] ±0.34	0.050*
48 h	0.48[a] ±0.30	0.21[a] ±0.10	0.43[a] ±0.20	0.21[a] ±0.10	0.200
72 h	0.30[a] ±0.12	0.25[a] ±0.26	0.32[a] ±0.10	0.24[a] ±0.30	0.900
1 week	0.35[c] ±0.10	0.27[d] ±0.05	0.43[c] ±0.08	0.46[c] ±0.07	0.951
2 weeks	4.31[a] ±0.74	4.13[a] ±0.75	4.37[a] ±0.76	4.15[a] ±0.68	0.864
3 weeks	4.92[a] ±0.67	4.68[a] ±0.94	4.96[a] ±0.68	4.72[a] ±0.93	0.797
6 weeks	3.75[a] ±0.35	3.58[b] ±0.52	3.75[a] ±0.35	3.58[a] ±0.52	0.909
p-value	≤0.001*	≤0.001*	≤0.001*	≤0.001*	–

Data presented as *M ±SD*.
* statistically significant; different letters in superscript denote statistically significant differences (pairwise comparison with the Bonferroni correction).

Table 3. Descriptive statistics and the test of significance for the rate of nano-chitosan release [µmf/cm²] for the tested groups at different investigated time intervals

Time point	Rate of nano-chitosan release [µmf/cm²]			p-value
	G2	G3	G4	
Initially	0.53[aB] ±0.01	1.09[aA] ±0.00	0.53[bB] ±0.01	≤0.001*
1 h	0.08[a] ±0.01	0.04[b] ±0.00	0.05[b] ±0.04	0.040*
24 h	0.60[b] ±0.00	0.85[a] ±0.00	0.60[b] ±0.00	0.010*
48 h	0.83[a] ±0.00	0.83[a] ±0.00	0.44[b] ±0.00	0.000*
72 h	0.35[b] ±0.00	0.35[b] ±0.00	0.94[a] ±0.00	0.000*
1 week	0.30[bB] ±0.00	0.30[bB] ±0.00	0.78[aA] ±0.00	≤0.001*
2 weeks	0.30[bB] ±0.00	0.30[bB] ±0.00	0.78[aA] ±0.00	≤0.001*
3 weeks	0.01[c] ±0.00	0.01[c] ±0.00	0.01[c] ±0.00	0.992
6 weeks	0.03[cA] ±0.00	0.03[cA] ±0.00	0.01[cB] ±0.00	≤0.001*
p-value	≤0.001*	≤0.001*	≤0.001*	–

Data presented as M ±SD.
* statistically significant; different uppercase letters in superscript denote statistically significant differences with regard to rows, and different lowercase letters in superscript denote statistically significant differences with regard to columns (pairwise comparison with the Bonferroni correction).

The minimum rate of nano-chitosan release was recorded after 6 weeks from immersion, with significant differences in regard to different immersion periods (0.03 ±0.00 µmf/cm², 0.03 ±0.00 µmf/cm² and 0.01 ±0.00 µmf/cm² for G1, G2 and G3, respectively.

Antibacterial test

The results of the antibacterial test for *S. aureus* for different investigated groups are presented in Table 4 and Fig. 3. Statistical analysis revealed the highest value of the in-

Table 4. Descriptive statistics and the test of significance for the antibacterial test before and after the addition of nano-chitosan to the glass-ionomer cement (GIC) for different investigated groups

Groups	µSBS [MPa]	p-value
G1	5.56 ±0.63[d]	
G2	10.20 ±0.83[a]	0.001*
G3	8.70 ±0.62[b]	
G4	6.08 ±0.81[c]	

Data presented as M ±SD.
* statistically significant; different letters in superscript denote statistically significant differences (paired samples t test).
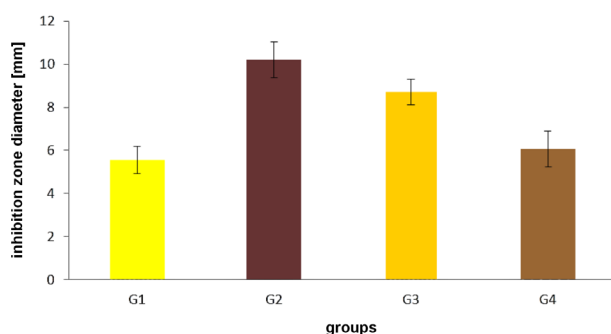
Fig. 3. Histogram showing the size of the inhibition zone against *Streptococcus aureus* among different investigated groups

hibition zone for G2 (10.20 ±0.83 mm), followed by G3 (8.70 ±0.62 mm). Groups G1 and G4 had the smallest inhibition zones of 5.56 ±0.63 mm and 6.08 ±0.81 mm, respectively.

Discussion

Glass-ionomer cements are extensively utilized in dentistry for restoring and preventing WSLs because of their unique characteristics. Nano-chitosan is one of the recent additives hybridized with traditional GICs to improve their antibacterial activity and remineralization potential, without sacrificing their mechanical and physical properties, fluoride release, and adhesion to hard tooth structures.[8]

According to certain theories, nano-chitosan particles, which were utilized in this study, may interact with the medium more effectively than larger chitosan particles because of their greater surface area and charge density. Moreover, these properties encourage the usage of nano-chitosan as a preventive and therapeutic agent to manage dental caries due to its powerful antibacterial activity against oral biofilm, particularly *S. mutans*.[29,30] Therefore, the present study aimed to investigate the effect of modifying the liquid phase of a traditional GIC with different nano-chitosan volume content on µSBS to enamel at the WSL area, fluoride and nano-chitosan release, and antibacterial properties.

In the present study, the µSBS test was used as a substitute for the conventional shear test in agreement with Banomyong et al., who replaced the blade with a looped orthodontic wire, as stress distribution in the µSBS test is more concentrated at the interface, reducing the risk of cohesive failure in materials that do not have reliable adhesion at the interface.[31] Generally, similar µSBS has been reported with regard to dentin when comparing

chitosan-modified and traditional GICs.[32–34] Moreover, GIC bonding to enamel is an important aspect in determining the strength of the bond between the material and WSLs. Nevertheless, there are very limited published works available in the dental literature assessing the bonding of modified GICs to enamel.[32]

Therefore, in this study, the 1st objective was to examine the influence of modifying GIC liquid with nano-chitosan on μSBS as compared to an unmodified GIC. Any GIC modification must not compromise its capacity to adhere to enamel and/or dentin.[33] The 1st null hypothesis of this study is rejected, since the addition of 50% v/v nano-chitosan to a traditional GIC improved the μSBS of the GIC to enamel WSLs. In the current study, the μSBS test revealed that the GIC modified via the incorporation of 50% nano-chitosan (G2) resulted in slightly, but non-significantly increased values as compared to the traditional GIC (G1) (4.1 ±0.94 MPa and 3.9 ±1.30 MPa, respectively) (Table 1, Fig. 2). These results are in agreement with Karthick et al., who found that the μSBS of 50% v/v chitosan-modified GIC was higher than in the case of a traditional GIC.[34] It might be due to the presence of polar and ionic reactivity between the carboxylic groups of PAA and hard tooth surfaces. More PAA chains would diffuse into enamel to displace the phosphate and calcium ions from hydroxyapatite crystals, since chitosan is a powerful chelating agent.[33,34] In their recent study, Patel et al. notices that chitosan chains include several acetamide and hydroxyl groups that can bind with the hydroxyl groups of GIC particles and the carboxyl groups of PAA by hydrogen bonding interaction.[11] The network produced by chitosan and PAA around inorganic GIC particles may lower the interfacial tension among GIC components, which can enhance or maintain its mechanical characteristics. One may postulate that NPs with lower particle sizes may operate as fillers after the GIC has set, filling the empty microscopic gaps between the larger unreacted GIC glass particles and providing extra bonding sites for the polyacrylic polymer, thereby reinforcing the GIC set material.[35]

Conversely, when nano-chitosan content increased to 150% v/v (G4), μSBS decreased (2.2 ±0.62 MPa) (Table 1, Fig. 2). This might be due to the segregation of chitosan chains with increasing chitosan content; they interact with each other, and no longer with PAA and/or GIC particle surfaces in addition to tooth calcium and phosphorus. These results are consistent with those of previous studies by Petri et al.,[30] Ibrahim et al.[32] and Karthick et al.[34] In addition, it is possible that increasing nano-chitosan content to 150% v/v (G4) results in the mechanical weakening of GIC rather than the failure of chemical bond formation to hard tooth structures.[32] This might be attributed to the agglomeration of NPs and the occupation of all free charges, which can lead to a rapid increase in viscosity that results in dimensional changes in the set cement. Such changes may lead to the formation of cracks and

the loss of marginal adaptation due to the development of contraction stress on the tooth wall. Consequently, water sorption increases, which in turn decreases bond strength.[36] Although GIC bonding to enamel is crucial in determining the bond strength of the material, enamel and nano-chitosan-modified GICs show adhesive bonding failure, with no cohesive failure.[33]

One of the milestones in suppressing further growth of caries at WSLs was implementing fluorides as an anti-cariogenic agent. Fluoride has been shown to reduce demineralization and promote remineralization. Furthermore, secondary caries was observed to be greatly reduced. The anti-cariogenic impact of fluoride-releasing restoration is determined by the amount and duration of fluoride release.[1] Therefore, in this study, the 2nd objective was to examine the influence of modifying GIC liquid with nano-chitosan on fluoride and nano-chitosan release, and to compare it to a traditional GIC. The 2nd null hypothesis of this study is partially accepted, since the addition of high concentrations of nano-chitosan to a traditional GIC increased fluoride release as a function of time.[10]

The results of the present study showed a significantly greater rate of fluoride release by traditional GIC samples (G1) as compared to the nano-chitosan-modified GIC groups (G2–G4) (Table 2). The G1 samples had an initial release rate of 1.70 ±0.21 μmf/cm^2 and a rate of 0.70 ±0.30 μmf/cm^2 after 24 h. These results could be due to the lower pH value initially and after 24 h. Additionally, these findings resemble the "burst effect" from a traditional GIC described by De Almeida Brandão Guglielmi et al.[4] and Panpisut and Toneluck,[21] who found that an acid-base reaction causes early fluoride release from a GIC, with the amount of fluoride released proportional to the fluoride concentration in the material. The "burst effect" is characteristic of large concentrations of fluoride being released within the first 2 days.

After 24 h, G4 recorded the highest fluoride release rate (0.54 ±0.34 μmf/cm^2), followed by G3 (0.40 ±0.90 μmf/cm^2). Group G2 recorded the lowest fluoride release rate (0.30 ±0.20 μmf/cm^2) (Table 2). The addition of nano-chitosan may have increased the burst action of the released fluoride.[10,30] These results are in agreement with Kumar et al., who found the addition of nano-chitosan to a GIC to have a catalytic effect on fluoride release, thereby aiding the rapid diffusion of fluoride through the GIC matrix.[10] In addition, the entropic gain associated with fluoride emission could explain the catalytic action. Since the entropy associated with small ions diffusing into the medium is always greater than that associated with free macromolecules in the solution, the adsorption of a polymer that causes the displacement of small ions always moves the system to a more favorable energy condition.[30] According to Kim et al., GICs containing chitosan increase the water sorption value and increase the diameter of the reacted structure.[37] Consequently, the increased friction of the

electrostatic charge with repulsive action leads to temporarily stable conditions for fluoride ion bursting. The minimum rate of fluoride release was recorded in the period between 48 h and 1 week from immersion for all the investigated groups (Table 2). These findings can be explained by the fact that the fluoride level is controlled by diffusion that is accompanied by a reduced concentration, which is linear to the square root of time. Therefore, the initial high concentration of fluoride rapidly decreased after 48 h before dropping quickly during the 1st week and eventually stabilizing. These findings are in accordance with Panpisut and Toneluck[21] and Baroudi et al.[38]

The addition of nano-CS to a traditional GIC enhanced its antibacterial activity, positively affecting the anti-cariogenic effect and antibiofilm capabilities. The antibacterial activity of nano-chitosan is linked to its molecular weight and viscosity. Low- and medium-molecular-weight nano-chitosan with low and medium viscosity infiltrates into the bacterial cell and attaches to the microbe DNA, preventing transcription and subsequent translation, and causing bacterial death.[35] The *S. aureus* strain is one of the most important Gram-positive pathogen involved in dental caries.[28] Therefore, it was selected as a model bacterial strain examined in this study. The 3rd hypothesis of this study that an increased nano-chitosan concentration would enhance the antibacterial effect in comparison with a traditional GIC was not confirmed.

Among all groups, the greatest inhibition zone against *S. aureus* was recorded for G2 (10.20 ±0.83 mm). Group G4 revealed the smallest zone of inhibition (6.08 ±0.81 mm) (Table 4, Fig. 3). This might be attributed to the lesser segregation of the polymer chain (nano-chitosan) and a high amount of free, unattached negative charges in G2. According to Ibrahim et al.[35] and Thurnheer and Belibasakis,[39] the unattached charges can bond easily to the positive charges of the bacterial cell wall, penetrating the DNA of *S. aureus* and damaging the hydrophobic bacterial cell wall. Since Gram-positive bacteria have a simple cell wall – only a stiff peptidoglycan layer with large holes present – it is easy for foreign substances to enter the cells. Hence, the interaction between the attached polymers and the charged membrane facilitates the destruction of microbes. Moreover, Zeimaran et al. demonstrated a network with plenty of pores inside the peptidoglycan layer of the cell wall of *S. aureus*.[40] So, nano-chitosan could enter the cell without difficulty via penetrating through the cell wall.

On the other hand, increasing the amount of nano-chitosan decreased the number of negative charges due to the segregation of the polymer chain.[30,32,33] Hence, the inhibition effect on the bacterial cell wall decreased, as shown in G3 and G4 (Table 4, Fig. 3). Even though G3 and G4 recorded the highest amount of fluoride release, they showed a weaker antibacterial effect as compared to G2. This might be attributed to the nature of the released fluoride. The fluoride ion is bonded to the cationic groups of chitosan. Fluoride ions block free sites on chitosan. These results are supported by a higher amount of nano-chitosan release for those groups. The synchronization of fluoride and nano-chitosan release depressed the antibacterial effect. These findings are in agreement with previous research performed by Ibrahim et al.[35] and Thurnheer and Belibasakis.[39]

Limitations

This in vitro research has certain limitations. The oral cavity is constantly subjected to dynamic stresses, mastication wear and pH changes caused by consuming various meals and beverages. Further research is needed to assess nano-chitosan-modified GICs in such dynamic settings, utilizing a larger sample size and a longer observation period. In addition, from a geometrical standpoint, the disks utilized do not entirely resemble a dental restoration.

Conclusions

Within the limitations of this study, the following conclusions can be drawn:

The addition of nano-chitosan (v/v) to a traditional GIC up to 50% of PAA liquid has been shown to increase the μSBS of the affected enamel surfaces and the antibacterial effect against *S. aureus*, which could potentially help to reduce the risk of secondary caries after restoring the enamel defect.

The synergistic effect of the nano-chitosan-modified GIC 150% (v/v) of PAA liquid significantly increased fluoride release, which might improve remineralization capacity while adversely impacting the antibacterial effect.

Ethics approval and consent to participate

The present study was approved by the Medical Research Ethics Committee (ref. No. 03/06/2022) at the Faculty of Oral and Dental Medicine, Nahda University, Beni Suef, Egypt.

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

Heba Abdelhamid Shalaby https://orcid.org/0000-0002-1665-1962
Nofal Khamis Soliman https://orcid.org/0000-0002-1731-0449
Khaled Wagih Al-Saudi https://orcid.org/0000-0002-5987-703X

References

1. Fricker JP. Therapeutic properties of glass-ionomer cements: Their application to orthodontic treatment. *Aust Dent J*. 2022;67(1):12–20. doi:10.1111/adj.12888
2. Knösel M, Alvarez RV, Blanck-Lubarsch M, Helms HJ. Comparison of potential long-term costs for preventive dentistry treatment of post-orthodontic labial versus lingual enamel cavitations and esthetically relevant white-spot lesions: A simulation study with different scenarios. *Head Face Med*. 2019;15(1):22. doi:10.1186/s13005-019-0204-x
3. Malik Z, Butt DQ, Butt ZQ, et al. Evolution of anticariogenic resin-modified glass ionomer cements. *ChemBioEng Rev*. 2021;8(4):326–336. doi:10.1002/cben.202100005
4. De Almeida Brandão Guglielmi C, Bissoto Calvo AF, Tedesco TK, Mendes FM, Raggio DP. Contact with fluoride-releasing restorative materials can arrest simulated approximal caries lesion. *J Nanomater*. 2015:259753. doi:10.1155/2015/259753
5. Moheet IA, Luddin N, Ab Rahman I, Kannan TP, Abd Ghani NR, Masudi SM. Modifications of glass ionomer cement powder by addition of recently fabricated nano-fillers and their effect on the properties: A review. *Eur J Dent*. 2019;13(3):470–477. doi:10.1055/s-0039-1693524
6. Gurgan S, Vural UK, Atalay C, Tassery H, Miletic I, Gurpinar SS. Antibacterial activity and biofilm inhibition of new-generation hybrid/fluoride-releasing restorative materials. *Appl Sci*. 2022;12(5):2434. doi:10.3390/app12052434
7. Ching HS, Luddin N, Kannan TP, Ab Rahman I, Abdul Ghani NR. Modification of glass ionomer cements on their physical-mechanical and antimicrobial properties. *J Esthet Restor Dent*. 2018;30(6):557–571. doi:10.1111/jerd.12413
8. Sayed RS, Shalaby HA, Hussein Ali YF, Soliman NK. Surface durability of newly glass ionomer cement modified with chitosan. *Researcher*. 2018;10(5):75–83. doi:10.7537/marsrsj100518.10
9. Avcu E, Baştan FE, Abdullah HZ, Ur Rehman MA, Avcu YY, Boccaccini AR. Electrophoretic deposition of chitosan-based composite coatings for biomedical applications: A review. *Prog Mater Sci*. 2019;103:69–108. doi:10.1016/j.pmatsci.2019.01.001
10. Kumar RS, Ravikumar N, Kavitha S, et al. Nanochitosan modified glass ionomer cement with enhanced mechanical properties and fluoride release. *Int J Biol Macromol*. 2017;104(Pt B):1860–1865. doi:10.1016/j.ijbiomac.2017.05.120
11. Patel A, Dhupar JK, Jajoo SS, Shah P, Chaudhary S. Evaluation of adhesive bond strength, and the sustained release of fluoride by chitosan-infused resin-modified glass ionomer cement: An in-vitro study. *Int J Clin Pediatr Dent*. 2021;14(2):254–257. doi:10.5005/jp-journals-10005-1943
12. Bhat S, Lolayekar N, Bhat SV. Disinfection methods of extracted human teeth. *J Oral Health Comm Dent*. 2007;1(2):27–29. doi:10.5005/johcd-1-2-27
13. Abo-Alazm EA, Safy RK. Impact of immediate dentin sealing using universal adhesive under simulated pulp pressure on micro-tensile bond strength of indirect resin composite restorations and dentin permeability. *Eur J Dent*. 2022;16(3):536–542. doi:10.1055/s-0041-1735442
14. Alqahtani MA, Almosa NA, Alsaif KA, Alsaif NM, Aljaser YJ. Effect of topical fluoride application and diode laser-irradiation on white spot lesions of human enamel. *Saudi Dent J*. 2021;33(8):937–943. doi:10.1016/j.sdentj.2021.08.007
15. Sattabanasuk V, Shimada Y, Tagami J. Bonding of resin to artificially carious dentin. *J Adhes Dent*. 2005;7(3):183–192. PMID:16240958.
16. Mobarak EH, Ali N, Daifalla LE. Microshear bond strength of adhesives to enamel remineralized using casein phosphopeptide agents. *Oper Dent*. 2015;40(5):E180–E188. doi:10.2341/13-220-L
17. Elnawawy HM, Morsi TS, El-Askary FS. The effect of different bonding protocols on the repair microshear bond strength of water-stored CAD/CAM resin composite. *J Adhes Sci Technol*. 2018;32(20):2254–2267. doi:10.1080/01694243.2018.1474563
18. Ivanoff CS, Hottel TL, Garcia-Godoy F. Influence of a cylindrical crosshead on shear bond testing of composite–tooth interfaces. *Dent Mater J*. 2018;37(6):865–873. doi:10.4012/dmj.2017-348
19. International Organization for Standardization (ISO). ISO 9917-1:2007: Powder/liquid acid-based cements. 2007. International Organization for Standardization, Geneva, Switzerland.
20. Agob JN, Aref NS, El Saeid Al-Wakeel E. Effect of casein phosphopeptide-amorphous calcium phosphate on fluoride release and micro-shear bond strength of resin-modified glass ionomer cement in caries-affected dentin. *Restor Dent Endod*. 2018;43(4):e45. doi:10.5395/rde.2018.43.e45
21. Panpisut P, Toneluck A. Monomer conversion, dimensional stability, biaxial flexural strength, and fluoride release of resin-based restorative material containing alkaline fillers. *Dent Mater J*. 2020;39(4):608–615. doi:10.4012/dmj.2019-020
22. Bueno LS, Silva RM, Magalhães AP, et al. Positive correlation between fluoride release and acid erosion of restorative glass-ionomer cements. *Dent Mater*. 2019;35(1):135–143. doi:10.1016/j.dental.2018.11.007
23. Shah A, Ashames AA, Buabeid MA, Murtaza G. Synthesis, in-vitro characterization and antibacterial efficacy of moxifloxacin-loaded chitosan-pullulan-silver-nanocomposite films. *J Drug Deliv Sci Technol*. 2020;55:101366. doi:10.1016/j.jddst.2019.101366
24. Ali GW, El-Hotaby W, Hemdan B, Abdel-Fattah WI. Thermosensitive chitosan/phosphate hydrogel-composites fortified with Ag versus Ag@Pd for biomedical applications. *Life Sci*. 2018;194:185–195. doi:10.1016/j.lfs.2017.12.021
25. Foong LK, Foroughi MM, Mirhosseini AF, et al. Applications of nano-materials in diverse dentistry regimes. *RSC Adv*. 2020;10(26):15430–15460. doi:10.1039/d0ra00762e
26. Moghaddam SC, Negahdari R, Sharifi S, Dizaj SM, Torab A, Rezaei Y. Preparation and assessment of physicochemical possessions, solubility and antimicrobial properties of dental prosthesis glass ionomer cement containing curcumin nanocrystals. *J Nanomater*. 2022:1229185. doi:10.1155/2022/1229185
27. Nomura R, Morita Y, Matayoshi S, Nakano K. Inhibitory effect of surface pre-reacted glass-ionomer (S-PRG) eluate against adhesion and colonization by *Streptococcus mutans*. *Sci Rep*. 2018;8(1):5056. doi:10.1038/s41598-018-23354-x
28. Sundeep D, Kumar TV, Rao PS, Ravikumar RV, Krishna AG. Green synthesis and characterization of Ag nanoparticles from *Mangifera indica* leaves for dental restoration and antibacterial applications. *Prog Biomater*. 2017;6(1–2):57–66. doi:10.1007/s40204-017-0067-9
29. Sharafeddin F, Jowkar Z, Bahrani S. Comparison between the effect of adding microhydroxyapatite and chitosan on surface roughness and microhardness of resin modified and conventional glass ionomer cements. *J Clin Exp Dent*. 2021;13(8):e737–e744. doi:10.4317/jced.55996
30. Petri DF, Donegá J, Benassi AM, Bocangel JA. Preliminary study on chitosan modified glass ionomer restoratives. *Dent Mater*. 2007;23(8):1004–1010. doi:10.1016/j.dental.2006.06.038
31. Banomyong D, Palamara JE, Burrow MF, Messer HH. Effect of dentin conditioning on dentin permeability and micro-shear bond strength. *Eur J Oral Sci*. 2007;115(6):502–509. doi:10.1111/j.1600-0722.2007.00483.x
32. Ibrahim MA, Neo J, Esguerra RJ, Fawzy AS. Characterization of antibacterial and adhesion properties of chitosan-modified glass ionomer cement. *J Biomater Appl*. 2015;30(4):409–419. doi:10.1177/0885328215589672
33. Debnath A, Kesavappa SB, Singh GP, et al. Comparative evaluation of antibacterial and adhesive properties of chitosan modified glass ionomer cement and conventional glass ionomer cement: An in vitro study. *J Clin Diagn Res*. 2017;11(3):ZC75–ZC78. doi:10.7860/JCDR/2017/25927.9593
34. Karthick A, Kavitha M, Loganathan SC, Malarvizhi D. Evaluation of microshear bond strength of chitosan modified GIC. *World J Med Sci*. 2014;10(2):169–173. doi:10.5829/idosi.wjms.2014.10.2.82184
35. Ibrahim MA, Priyadarshini BM, Neo J, Fawzy AS. Characterization of chitosan/TiO_2 nano-powder modified glass-ionomer cement for restorative dental applications. *J Esthet Restor Dent*. 2017;29(2):146–156. doi:10.1111/jerd.12282
36. Masaeli R, Ketabat F, Zandsalimi K. Microhardness and wear resistance of glass ionomer cements modified by chitosan and nano-hydroxyapatite. *J Dentomaxillofac Radiol Pathol Surg*. 2019;8(3):7–14. doi:10.32598/3dj.7.4.145

37. Kim J, Jung HM, Kim JY, Han J, Jin BH, Woo KM. Fluoride-releasing chitosan nanoparticles for prevention of dental caries. *Tissue Eng Regen Med*. 2011;8(4):93–98.

38. Baroudi K, Mahmoud RS, Tarakji B. Fluoride release of glass ionomer restorations after bleaching with two different bleaching materials. *Eur J Dent*. 2013;7(2):196–200. doi:10.4103/1305-7456.110171

39. Thurnheer T, Belibasakis GN. Effect of sodium fluoride on oral biofilm microbiota and enamel demineralization. *Arch Oral Biol*. 2018;89:77–83. doi:10.1016/j.archoralbio.2018.02.010

40. Zeimaran E, Pourshahrestani S, Djordjevic I, et al. Antibacterial properties of poly(octanediol citrate)/gallium-containing bioglass composite scaffolds. *J Mater Sci Mater Med*. 2015;27(18). doi:10.1007/s10856-015-5620-2

Morphological evaluation of the nasopalatine canal using cone beam computed tomography and its clinical implications for orthodontic miniscrew insertion

Marta Gibas-Stanek[A–F], Dominika Kościółek-Rudy[B–D,F], Klaudia Szumilas[B–D,F], Karolina Wojas-Hille[C–F], Małgorzata Pihut[E,F]

Institute of Dentistry, 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

Abstract

Background. The morphology of the nasopalatine canal is crucial in the planning of prosthetic restorations in the anterior region of the maxilla, as well as in the placement of orthodontic mini-implants.

Objectives. The aim of this study was to assess the morphology of the nasopalatine canal using cone beam computed tomography (CBCT) scans of patients from the University Dental Clinic in Krakow, Poland, to define the position of the canal in relation to common sites of palatal median microimplant placement, and to investigate potential correlations between the anatomy of the canal and age and gender of the patients.

Material and methods. A total of 120 CBCT images were used to assess the anatomy of the nasopalatine canal in 3 planes of space. The bone thickness anterior to the nasopalatine canal and the distance between the distal margin of Stenson's foramen and the predicted midpalatal microimplant position were also measured.

Results. The most frequently observed canal type in the coronal plane was the Y-shaped canal, which was present in 60.8% of patients. The nasopalatine canal was classified as cone-shaped in 31.7% of the scans, cylindrical in 28.3%, hourglass-shaped in 27.5%, and banana-shaped in 12.5%. The mean length of the nasopalatine canal was 11.58 mm. The mean width of the canal was 2.89 mm at the nasal fossa level, 1.94 mm in the middle, and 5.09 mm at the palatal level. The mean bone thickness anterior to the nasopalatine canal was 9.07 mm at the level of the nasal opening, 6.84 mm at the level of the oral opening, and 7.32 mm in the middle. The mean distance between the distal margin of Stenson's foramen and the predicted midpalatal microimplant position varied from 0 to 11.94 mm, with a mean of 2.49 mm.

Conclusions. Given the variety of nasopalatine canal forms and dimensions, detailed analysis of CBCT scans is essential prior to the placement of implants and microimplants.

Keywords: cone beam computed tomography (CBCT), nasopalatine canal, incisive canal dimensions

Introduction

The nasopalatine canal is an important anatomical structure situated in the middle of the maxilla behind the upper incisors. This bony conduit connects the oral and nasal cavities and contains neurovascular structures that provide sensation and vascular supply for neighboring areas.[1] In the oral cavity, the nasopalatine canal terminates as the incisive foramen, while the upper opening extends to the nasal cavity and is known as Stenson's foramen or the nasal foramen. Stenson's foramen is frequently located on both sides of the nasal septum.[2] The morphology of the nasopalatine canal is crucial in the planning of prosthetic restorations in the anterior region of the maxilla, primarily because the limited bone volume in this area affects the stability and osteointegration of dental implants.[3–6] In particular, it is of great importance to diagnose the pathology of the nasopalatine canal, as the most common nonodontogenic cysts in the oral cavity are nasolabial cysts.[7] In addition to these considerations for prosthetic and surgical treatment, the anatomy and position of the nasopalatine canal also determine orthodontic treatment planning. It was found that orthodontic tooth movement within the anterior part of the maxilla, particularly maxillary incisor retraction involving nasopalatine canal invasion, resulted in root resorption.[8,9]

The anterior palate is considered a valuable alternative for orthodontic mini-implant placement, as it offers the possibility of avoiding interference with roots and reduces the mini-implant failure rate on account of its good bone density.[10] However, according to Wilmes et al., there is a risk of penetrating the nasopalatine canal when a mini-screw is inserted in the median position, which may affect the stability of the mini-implant.[10] In particular, penetration of the miniscrew into the nasopalatine bundle may result in anchorage loss, palatal mucosa impingement, or palatal bone loss.[11]

Although the anatomical conditions of the nasopalatine canal have already been studied,[12–15] a more detailed analysis of the anatomical structures of the anterior maxilla is required due to the increasing popularity of bone-anchored orthodontic appliances. The objective of the present study was to assess the morphology of the nasopalatine canal using cone beam computed tomography (CBCT) scans of patients from the University Dental Clinic in Krakow, Poland. The obtained data was used to define the position of the nasopalatine canal in relation to the most common sites of palatal median microimplant placement and to investigate the correlations between the anatomy of the canal and age and gender of the patients.

Material and methods

The sample size was determined with the use of a sample size calculator. The results indicate that a sample size of 116 is sufficient to detect differences with a 5% margin of error, a 90% confidence level and a statistical power of 80%. The final number of patients included in the study was greater than the estimated number.

The data for this cross-sectional study was obtained from CBCT scans of the maxilla of patients at the University Dental Clinic in Krakow, Poland, performed for any reason between January 2018 and December 2021. The main indications for CBCT imaging were as follows: the assessment of unerupted third molars; the assessment of the dental implant site and the preparation of surgical guides; the diagnosis of pathologies in the paranasal sinuses; the diagnosis of periapical lesions and periodontal bone loss; planning orthognathic surgeries; and the assessment of bone thickness prior to orthodontic microimplant insertion. The study focused on 120 consecutively selected CBCT images (60 men and 60 women) that met the following inclusion criteria: age >8 years; the presence of upper central incisors; the presence of upper first premolars; the absence of pathologies in the anterior part of the maxilla (clefts, cysts, supernumerary teeth, impacted teeth); and the absence of maxillofacial syndromes. Images exhibiting artifacts were excluded from the study.

All CBCT scans were obtained using orthopantomography (OP 3D Pro; KaVo, Biberach an der Riß, Germany). The average parameters were as follows: field of view: 130 mm × 150 mm; average exposure time: 8.5 s; average scanning time: 39 s; and average voxel size: 380 µm.

All images were analyzed by a single trained and experienced senior postgraduate trainee in orthodontics. To determine intra-examiner error, repetitive measurements were conducted on 10 randomly selected scans over a 3-week interval to ensure reliability and to exclude learning bias. For the analysis of the CBCT images, a medical diagnostic monitor (RadiForce MX215; EIZO Inc., Cypress, USA) and an InVivo Dental Viewer software (Anatomage, Inc., Santa Clara, USA) were used.

The anatomy of the nasopalatine canal was evaluated in 3 planes of space:
- the axial (horizontal) plane, passing through the right and left orbital points and right and left porion points;
- the coronal plane, passing through the right and left porion points, perpendicular to the axial plane;
- the sagittal plane, passing through the nasion point, perpendicular to the axial and frontal planes.

In the coronal plane, each nasopalatine canal was classified according to its shape using the classification proposed by Bornstein et al.[16]:
- type A – single canal;
- type B – double canal;
- type C – Y-shaped canal.

A graphical representation of the various shapes of the nasopalatine canal in the coronal slices is presented in Fig. 1.
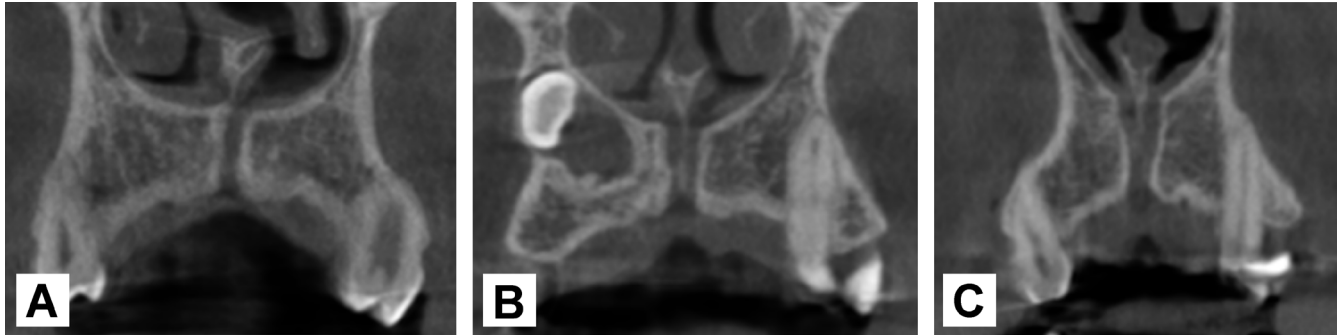
Fig. 1. Anatomy of the nasopalatine canal in the coronal plane

A. Single canal; B. Double canal; C. Y-shaped canal.

In the sagittal plane, each nasopalatine canal was classified according to its shape, as follows:
- cylindrical (labial and palatal walls are parallel);
- banana-shaped (labial and palatal walls are parallel, the canal is curved);
- cone-shaped (increasing anteroposterior dimension from the nasal opening to the oral opening);
- hourglass-shaped (the narrowest anteroposterior dimension is in the middle);
- spindle-shaped (the widest anteroposterior dimension is in the middle).

A graphical representation of the various shapes of the nasopalatine canal in the sagittal slices is presented in Fig. 2.

In addition, multiple measurements were taken from the sagittal slice images. The nasopalatine canal length, anteroposterior diameter, and the distance between the buccal wall of the nasopalatine canal and the facial surface of the buccal bone plate were all measured at 3 different levels:
- upper level (at the level of the nasal opening);
- middle level (in the middle of the nasopalatine canal);
- lower level (at the level of the oral opening).

A graphical representation of the nasopalatine canal measurements made in the sagittal slices is presented in Fig. 3.

The measurements obtained from the sagittal slices were further analyzed to assess their correlation with sex and age. In the sagittal view, the X-distance, representing the distance between the distal margin of Stenson's foramen and the predicted midpalatal microimplant position, was also measured (Fig. 4).

In the axial plane, the number of oral openings (incisive foramina) and the number of nasal openings (Stenson's foramina) were counted.

Fig. 3. Nasopalatine canal measurements performed in the sagittal plane

a – upper width of the nasopalatine canal; b – middle width of the nasopalatine canal; c – lower width of the nasopalatine canal; d – length of the nasopalatine canal; e – distance between the buccal wall of the nasal opening of the nasopalatine canal and the buccal bone plate; f – distance between the buccal wall of the nasopalatine canal and the buccal bone plate in the middle of the canal; g – distance between the buccal wall of the oral opening of the nasopalatine canal and the buccal bone plate.
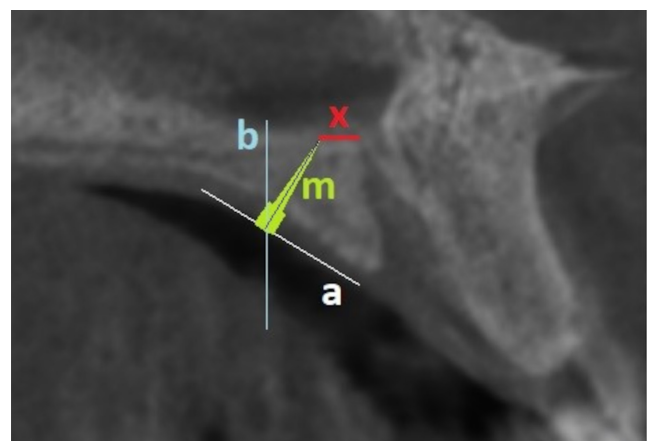
Fig. 4. Cone beam computed tomography (CBCT) image of the sagittal plane presenting the midpalatal microimplant placement technique and measuring the distance between the microimplant tip and the nasal opening of the nasopalatine canal

a – line parallel to the palatal mucosa; b – line passing through the middle of the first upper premolars; m – typical positioning of the midpalatal microimplant (insertion angle perpendicular to the palatal mucosa, insertion point at the level of the first maxillary premolars); x – distance between the tip of the microimplant and the posterior wall of Stenson's foramen.

Fig. 2. Classification of the nasopalatine canal in the sagittal plane

A. Cylindrical; B. Banana-shaped; C. Cone-shaped; D. Hourglass-shaped; E. Spindle-shaped.

The study was approved by the Bioethics Committee of Jagiellonian University, Krakow, Poland (approval No. 1072.6120.132.2020).

Statistical analysis

The data analysis was conducted using R software, v. 4.2.1 (R Foundation for Statistical Computing, Vienna, Austria). The normality of the distribution of the various groups was tested using the Shapiro–Wilk test. The concordance of measurements of quantitative variables was assessed using the intraclass correlation coefficient (ICC) type 2 (according to the classification of Shrout and Fleiss).[17] The χ^2 test (with Yates' correction for 2 × 2 tables) was employed to compare qualitative variables across the groups. When contingency tables exhibited low values, Fisher's exact test was used. The Mann–Whitney test was employed to compare quantitative variables between the 2 groups. The relationship between 2 quantitative variables was evaluated using Spearman's correlation coefficient. The Kruskal–Wallis test was used to compare quantitative variables across more than 2 groups. The significance level for all statistical tests was set at 0.05.

Results

A total of 120 consecutively selected CBCT scans of the maxilla that met the aforementioned criteria were included in this study. The scans were obtained from 60 women (mean age: 34.1 years) and 60 men (mean age: 32.2 years). The age of all patients ranged from 11 to 76 years.

Ten randomly selected scans were subjected to repeated measurements within a 3-week interval. The quantitative variable assessment demonstrated excellent and good accordance between the first and second measurements, as indicated by the ICC type 2 (Table 1).

The most frequently observed canal type in the coronal plane was the Y-shaped canal (60.8% of patients), followed by single (31.7%) and double canals (7.5%) (Table 2). The morphological variations assessed in the sagittal plane are presented in Fig. 5. The nasopalatine canal was classified as cone-shaped in 31.7% of the scans, cylindrical in 28.3%, hourglass-shaped in 27.5%, and banana-shaped in 12.5% of cases. No canal was classified as spindle-shaped, thus it was excluded from further consideration.

There was no statistically significant difference between the sexes when considering the shape of the nasopalatine canal in the coronal and sagittal planes (Fig. 6,7) (Table 2). Additionally, there was no statistically significant difference between the sexes in terms of the number of nasal and oral openings. In total, 91.7% of cases exhibited a single oral opening, while 8.3% displayed 2 openings. Taking into consideration the number of nasal openings of the nasopalatine canal, the most prevalent morphological variation was 2 openings (50.8%), followed by a single foramen (34.2%). The presence of 3 and 4 openings was observed in 14.2% and 0.8% of cases, respectively (Fig. 8). The aforementioned results are presented in Fig. 9.

Table 2. Comparison of the shape of the nasopalatine canal between men and women

Parameter		Female (n = 60)	Male (n = 60)	p-value (χ² test)
Canal shape in the coronal plane	single canal	17 (28.33)	21 (35.00)	
	double canal	5 (8.33)	4 (6.67)	0.771
	Y-shaped canal	38 (63.33)	35 (58.33)	
Canal shape in the sagittal plane	cylindrical	22 (36.67)	12 (20.00)	
	banana-shaped	8 (13.33)	7 (11.67)	0.135
	cone-shaped	18 (30.00)	20 (33.33)	
	hourglass-shaped	12 (20.00)	21 (35.00)	

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

Table 1. Intra-examiner error calculation

Parameter	1st measurement (M ±SD)	2nd measurement (M ±SD)	ICC	95% CI	Agreement (Cicchetti[18])	Agreement (Koo & Li[19])
Length [mm]	11.95 ±3.00	11.89 ±3.07	0.981	0.929–0.995	excellent	excellent
Upper width [mm]	3.72 ±2.97	3.55 ±2.84	0.980	0.927–0.995	excellent	excellent
Middle width [mm]	1.86 ±1.12	1.83 ±1.28	0.938	0.784–0.984	excellent	excellent
Lower width [mm]	4.18 ±1.25	4.52 ±1.46	0.849	0.519–0.960	excellent	good
Bone thickness (upper) (at the level of Stenson's foramen) [mm]	9.3 ±1.91	9.26 ±1.92	0.956	0.844–0.989	excellent	excellent
Bone thickness (middle) [mm]	7.77 ±1.46	7.99 ±1.69	0.857	0.549–0.962	excellent	good
Bone thickness (lower) (at the level of the oral opening) [mm]	6.74 ±0.98	6.68 ±1.34	0.845	0.519–0.959	excellent	good

M – mean; SD – standard deviation; ICC – intraclass correlation coefficient; CI – confidence interval.
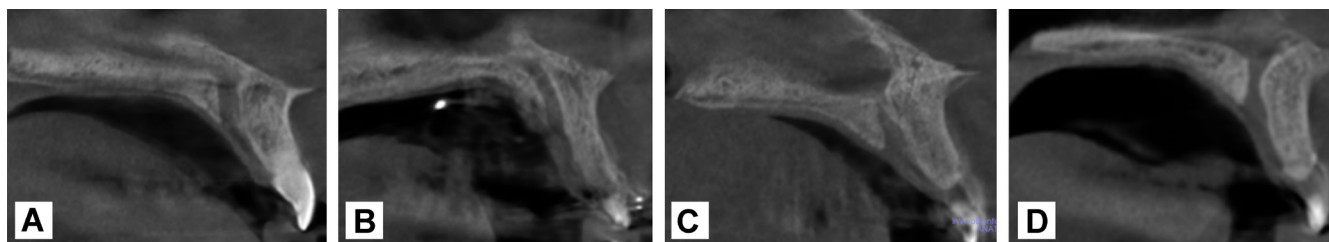
Fig. 5. Morphology of the nasopalatine canal in the sagittal plane
A. Cylindrical; B. Banana-shaped; C. Cone-shaped; D. Hourglass-shaped.
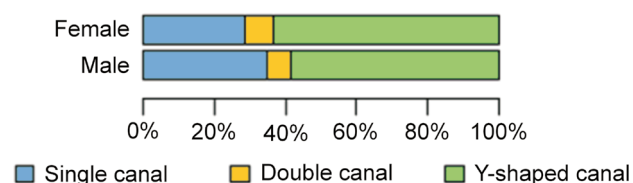
Fig. 6. Prevalence of the nasopalatine canal shape in men and women in the coronal plane
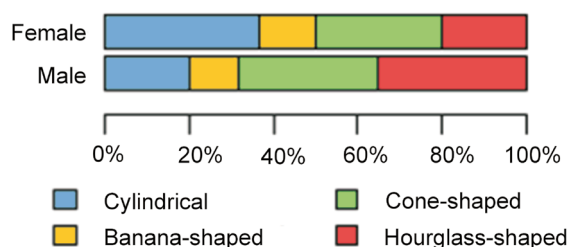
Fig. 7. Prevalence of the nasopalatine canal shape in men and women in the sagittal plane
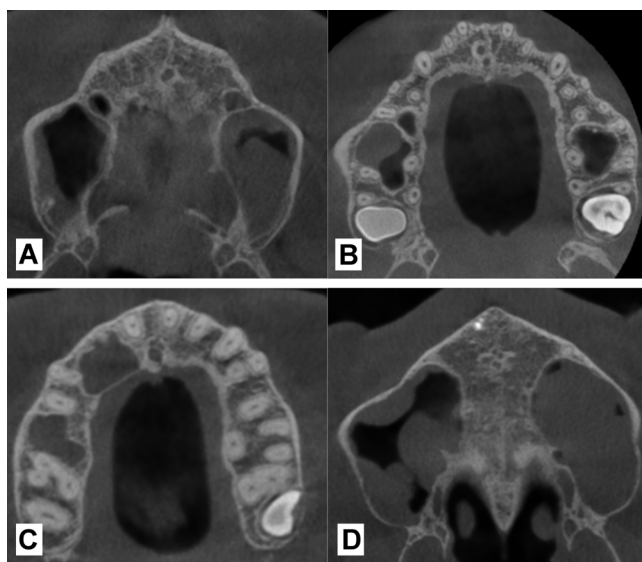
Fig. 8. CBCT images of Stenson's foramina in the axial plane
A. Single opening; B. Two openings; C. Three openings; D. Four openings.

The measured length of the nasopalatine canal ranged from 4.39 to 22.22 mm, with a mean length of 11.58 mm. The mean anteroposterior diameter of the canal at the nasal fossa level was 2.89 mm, the mean middle width was 1.94 mm, and the mean diameter at the palatal level was 5.09 mm. The mean bone thickness anter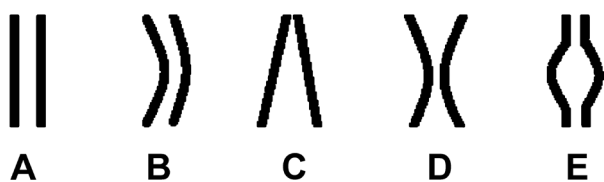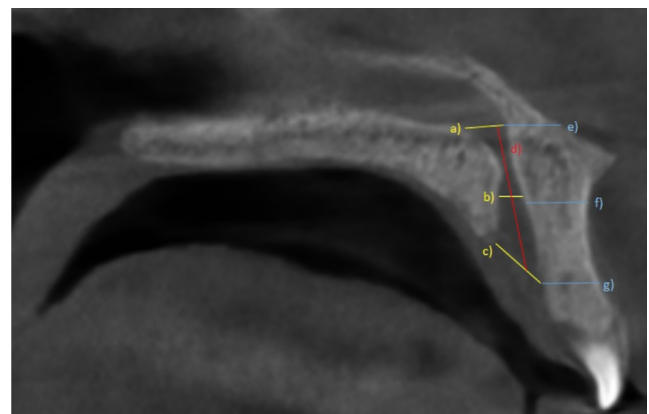ior to the nasopalatine canal was 9.07 mm at the level of the nasal opening, 6.84 mm at the level of the oral opening, and 7.32 mm in the middle.

The Mann–Whitney U test was used to compare the length of the nasopalatine canal, the width of the nasopalatine canal and the bone thickness in the anterior region of the maxilla between men and women. Significant differences were observed between the sexes for the mean length of the canal (2.39 mm longer in males), the mean upper width of the canal (0.58 mm wider in males), the mean upper bone thickness, and the mean middle bone thickness (0.79 mm and 0.53 mm thicker in males, respectively). The results of the measurements and comparisons between the sexes are presented in Table 3. No tested parameter demonstrated a correlation with the age of the patients ($p > 0.05$) (Table 4).

The X-distance varied from 0 to 11.94 mm, with a mean of 2.49 mm. There was no statistically significant difference between the sexes ($p > 0.05$) (Table 5). Additionally, no correlation was observed between the X-distance and the shape of the nasopalatine canal in the sagittal plane ($p > 0.05$) (Table 6).

Discussion

Considering the shape of the nasopalatine canal in the coronal plane,[19] the most prevalent canal type in our study was the Y-shaped canal (60.8% of patients), followed by single (31.7%) and double canals (7.5%). Similar results were reported by Bahşi et al., who observed a Y-shaped canal in 63.3% of cases, a single canal in 36% of cases, and a double canal in 0.7% of cases.[20] However, the results differ from those reported in an Iranian study,[21] where the Y-shaped canal was still the most common, but in only 46.46% of cases, followed by single (43.4%) and double canals (10%). In an analysis conducted by Bornstein et al. on Swiss patients, the most common form of the nasopalatine canal in the coronal plane was the single canal (45%), while the Y-shaped canal occurred in only 40% of scans.[16] Despite these variations, all studies confirmed that the double canal is the least common form of the nasopalatine canal.

Observations of the nasopalatine canal in the sagittal plane did not reveal any significant differences in
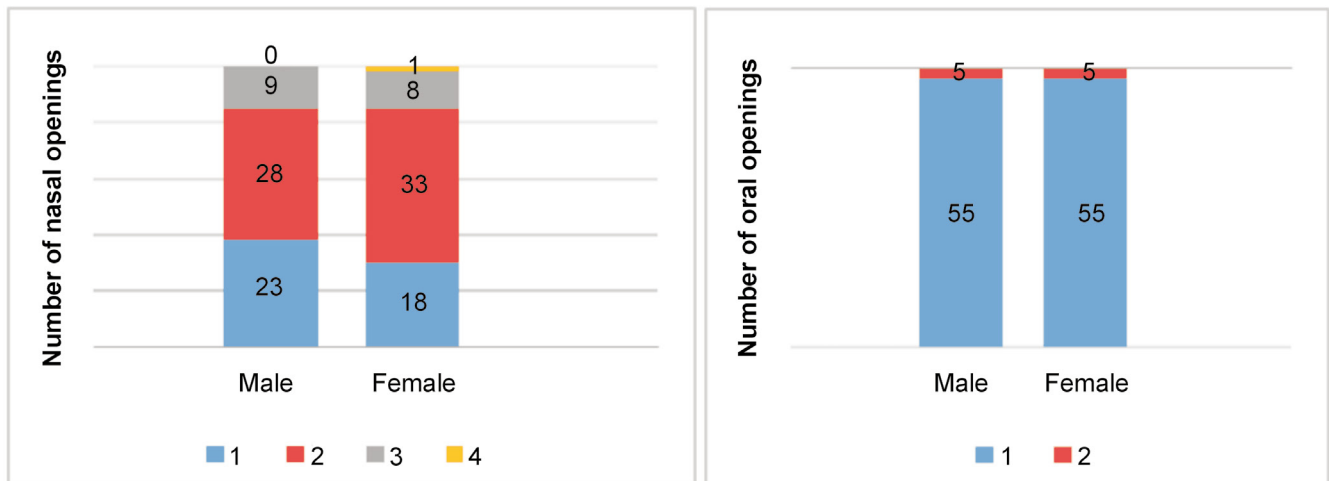
Fig. 9. Length and width of the nasopalatine canal in men and women

Table 3. Length and width of the nasopalatine canal and the thickness of the bone anterior to the nasopalatine canal between men and women

Parameter	Sex	n	M	SD	Me	Min	Max	Q1	Q3	p-value
Length [mm]	female	60	10.38	2.25	10.14	4.39	15.64	9.20	12.04	<0.001*
	male	60	12.77	3.59	12.89	4.97	22.22	10.30	14.82	
Upper width [mm]	female	60	2.60	1.59	2.02	0.54	7.56	1.43	3.41	0.042*
	male	60	3.18	1.81	2.79	0.68	8.89	1.84	4.01	
Middle width [mm]	female	60	1.90	1.07	1.67	0.56	5.09	1.13	2.49	0.343
	male	60	1.97	0.86	1.73	0.69	4.38	1.41	2.59	
Lower width [mm]	female	60	4.73	2.26	4.22	1.13	11.48	3.17	5.82	0.163
	male	60	5.45	2.86	4.94	0.56	13.65	3.23	7.01	
Bone thickness (upper) (at the level of Stenson's foramen) [mm]	female	60	8.67	1.72	8.59	5.51	14.18	7.43	9.53	0.006*
	male	60	9.46	1.94	9.36	4.94	14.85	8.53	10.57	
Bone thickness (middle) [mm]	female	60	7.05	1.35	6.94	3.91	10.36	6.26	7.73	0.027*
	male	60	7.58	1.41	7.50	4.94	11.43	6.52	8.45	
Bone thickness (lower) (at the level of the oral opening) [mm]	female	60	6.65	1.13	6.70	4.35	9.60	6.00	7.28	0.170
	male	60	7.03	1.25	6.84	4.35	9.92	6.20	7.72	

Me – median; Q1 – lower quartile; Q3 – upper quartile; * statistically significant (*p* ≤ 0.05, Mann–Whitney test).

Table 4. Comparison of measured parameters by patients' age

Parameter	Age	
	Spearman's correlation coefficient (*r*)	*p*-value
Number of oral openings	−0.097	0.293
Number of nasal openings	0.108	0.242
Length	0.017	0.856
Upper width	−0.064	0.489
Middle width	0.065	0.482
Lower width	0.172	0.061
Bone thickness (upper)	0.010	0.913
Bone thickness (middle)	−0.126	0.172
Bone thickness (lower)	−0.017	0.852

the prevalence between the cone-shaped canal (31.7% of patients), cylindrical canal (28.3%) and hourglass-shaped canal (27.5%). The banana-shaped canal was the least frequent, observed in 12.5% of patients. Our results are partially confirmed by the Turkish study,[20] which describes the nasopalatine canal as cylindrical in 28.7% of cases, hourglass-shaped in 26.7% and banana-shaped in 16%. Although there is disagreement in the prevalence of cone-shaped canal in the Turkish study (14.7%), this discrepancy can be attributed to methodological differences between the 2 studies. In the Turkish study, the nasopalatine canal was classified into 6 patterns, whereas in our study, it was classified into 4 patterns. The distribution of canal morphotypes found in the study by Thakur et al. suggests that the most common patterns in the Indian population are

Table 5. Correlation between the X-distance and the sex of the patients

Sex	X-distance [mm]							p-value (Mann–Whitney test)
	M	SD	Me	Min	Max	Q1	Q3	
Female	2.78	2.98	2.1	0	9.57	0	4.8	0.186
Male	2.20	3.14	0.0	0	11.94	0	3.9	

Table 6. Correlation between the X-distance and the shape of the nasopalatine canal in the sagittal plane

Canal shape in the sagittal plane	n	X-distance [mm]							p-value (Kruskal–Wallis test)
		M	SD	Me	Min	Max	Q1	Q3	
Cylindrical	27	2.46	3.10	0.00	0	8.83	0	4.69	0.923
Banana-shaped	15	2.07	2.83	0.00	0	7.51	0	4.43	
Cone-shaped	29	2.38	3.13	1.00	0	11.94	0	3.84	
Hourglass-shaped	29	2.80	3.20	2.31	0	10.18	0	4.44	

the cylindrical (39%) and funnel (or cone-shaped) types (31%).[22] There is a marked difference in the prevalence of spindle-shaped canals, which was 11% in their study compared to 0 cases in our study.

The nasopalatine canal was also classified according to the number of oral and nasal openings in the axial plane. In our study, 1 incisive foramen and 2 nasopalatine foramina were observed with greater frequency than the other configurations. Bahşi et al. determined that there was 1 oral opening in 88% of cases and 2 openings in 12%.[20] However, in the case of nasal openings, the most prevalent form was a single foramen (53.3%), followed by 2 foramina (44.7%) and 3 foramina (2%). Thakur et al. determined that most of their subjects had 2 Stenson's foramina (81%), with only 4% exhibiting a single foramen.[22] No data was provided concerning incisive foramina.

A detailed analysis of the nasopalatine canal revealed that the canal was longer in males (12.77 ±3.59 mm) than in females (10.38 ±2.25 mm). A similar difference was observed in the Indian study,[22] in which the mean length of the nasopalatine canal was determined to be 10.96 ±1.99 mm in males and 9.20 ±2.16 mm in females. This finding was further corroborated by Iranian (11.46 ±2.86 mm and 9.37 ±2.24 mm, respectively) and Turkish studies (13.68 ±2.73 mm and 11.43 ±2.78 mm, respectively).[21,23] Conversely, another Turkish study determined that the length of the nasopalatine canal was similar between the sexes (12.96 ±2.57 mm in males and 12.16 ±2.45 mm in females).[20] The review of 7 studies compared the mean length of the nasopalatine canal between the sexes and found that the nasopalatine canal was longer in males in all 7 studies, although the difference was statistically significant in only 4 reports.[20]

To describe the morphology of the nasopalatine canal, we also measured the diameter of the nasal and oral openings, as well as the width of the canal at its mid-level. The results indicated that the incisive foramen was wider than Stenson's foramen. However, while the width of the canal was greater in males at all levels, the difference was statistically significant only in the case of the upper width. While some studies have yielded similar results,[23] others have reported conflicting findings. Bahşi et al. observed a difference between the sexes in the width of the oral opening, but it is important to note that the level of significance was often close to the threshold value accepted for the purpose of the study ($p < 0.05$).[20] In contrast to our findings, Khojastepour et al. reported similar measurements for the diameters of the nasal and oral openings.[21] These discrepancies may be attributed to the differences in study methodology, including the distinct reference points chosen for the purpose of the study.

Considering the practical applications of knowledge regarding the morphology of the nasopalatine canal and adjacent areas, the thickness of the bone layer anterior to the nasopalatine canal is of particular importance. Implantation procedures require a detailed assessment of the implant site, and only adequate bone volume ensures therapeutic success. The results of this study indicate that the greatest bone thickness can be found at the level of Stenson's foramen (9.46 ±1.94 mm in males and 8.67 ±1.72 mm in females), while the lowest thickness is at the level of the oral opening of the nasopalatine canal (7.03 ±1.25 mm in males and 6.65 ±1.13 mm in females). It can be observed that the upper and middle widths depend on the sex of the patient. Gönül et al. found no significant difference between the sexes, but their results confirmed a decreasing bone width from the nasal opening toward the oral opening.[23] The study by Khojastepour et al. also found higher width values at the level of Stenson's foramen, but in contrast to our outcomes, it was the lower width of the buccal bone plate that was dependent on patient sex.[21]

Previous studies have provided evidence of a positive correlation between the size of the nasopalatine canal and the age of the patient.[24,25] Nevertheless, the dimensional changes seem to be strictly connected to the absence of central incisors. Consequently, the authors

attributed this phenomenon to atrophy of disuse, similar to maxillary sinus expansion after the loss of the posterior teeth. Gönül et al. observed a reduction in the distance between the incisive foramen and the facial facade of the buccal bone plate in the absence of 2 upper incisors.[23] Conversely, the results of another study, which only included patients whose upper central incisors were present, demonstrated a significant negative correlation between age and the width of the buccal bone anterior to the oral opening to the nasopalatine canal.[21] The same authors reported a significant positive correlation between age and the width of the incisive foramen. The results of our study indicated that no parameters were correlated with the age of the patients ($p > 0.05$). Bahşi et al. and Thakur et al. also found no significant relationship between age and the shape and size of the nasopalatine canal in the sagittal, coronal and axial planes.[20,22] However, these authors do not provide information regarding the presence or absence of the upper central incisors in the evaluated samples.

Given the prevalence of CBCT scans in orthodontic treatment planning and frequent consideration of skeletal anchorage as a component of orthodontic therapy, another purpose of this study was to assess the distance between the posterior wall of the nasopalatine canal and the predicted position of midpalatal microimplants (referred to as the X-distance in this study). This parameter indirectly describes the angulation of the nasopalatine canal in the sagittal plane. The more vertical the position of the nasopalatine canal, the greater the expected X-distance. To verify this hypothesis, we analyzed our data using the Kruskal–Wallis test. The results revealed that there was no correlation between the shape of the nasopalatine canal in the sagittal plane and the X-distance. The difference was also insignificant between males and females. Although the mean value of the X-distance was 2.49 mm and the maximum reached 11.94 mm, in 49% of scans, the distance from the posterior wall of Stenson's foramen to the predicted position of the miniscrew was equal to or less than 0 mm. This is of clinical significance and emphasises the critical need for a detailed evaluation of CBCT scans prior to microimplant placement procedures in each individual case in order to avoid nasopalatine canal penetration.

Limitations

The findings presented in this study are subject to several limitations. First, the sample size was relatively small. Second, the age range of the participants included in the study was considerable. Both growing and adult patients were included in the study. Despite the absence of parameters related to patient age (all parameters yielded $p > 0.05$ in Spearman's correlation coefficient analysis), it would be reasonable to design the research in a way that enables a comparison between growing patients and individuals

with completed craniofacial development. The authors of the present study did not identify any other studies describing the morphology of the nasopalatine canal in juvenile patients.

Conclusions

The nasopalatine canal exhibits considerable variation in size and morphology. In the cohort under investigation, the most frequently observed canal type was the Y-shaped canal, which exhibited 2 nasal openings and 1 oral opening. The most prevalent canal forms observed in the sagittal view were cone-shaped, cylindrical and hourglass-shaped. The length and width of the nasopalatine canal, as well as the anterior bone thickness in the front of the upper and middle parts of the nasopalatine canal, were found to be significantly greater in males. No parameters demonstrated a correlation with the age of the patient. Given the considerable variety of forms and dimensions of the nasopalatine canal, a detailed analysis of CBCT scans is essential prior to the placement of implants and microimplants.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee of Jagiellonian University, Krakow, Poland (approval No. 1072.6120.132.2020).

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

Marta Gibas-Stanek https://orcid.org/0000-0002-9837-0223
Dominika Kościółek-Rudy https://orcid.org/0000-0002-9616-328X
Klaudia Szumilas https://orcid.org/0000-0003-0831-3266
Karolina Wojas-Hille https://orcid.org/0000-0002-8798-6858
Małgorzata Pihut https://orcid.org/0000-0002-0239-4328

References

1. Lake S, Iwanaga J, Kikuta S, Oskouian RJ, Loukas M, Tubbs RS. The incisive canal: A comprehensive review. 2018;10(7):e3069. doi:10.7759/cureus.3069
2. Miwa Y, Asaumi R, Kawai T, Maeda Y, Sato I. Morphological observation and CBCT of the bony canal structure of the groove and the location of blood vessels and nerves in the palatine of elderly human cadavers. *Surg Radiol Anat.* 2018;40(2):199–206. doi:10.1007/s00276-017-1952-6
3. McCrea SJJ. Aberrations causing neurovascular damage in the anterior maxilla during dental implant placement. *Case Rep Dent.* 2017;2017:5969643. doi:10.1155/2017/5969643

4. Bodereau EF, Flores VY, Naldini P, Torassa D, Tortolini P. Clinical evaluation of the nasopalatine canal in implant-prosthetic treatment: A pilot study. *Dent J (Basel)*. 2020;8(2):30. doi:10.3390/dj8020030

5. Mraiwa N, Jacobs R, Van Cleynenbreugel J, et al. The nasopalatine canal revisited using 2D and 3D CT imaging. *Dentomaxillofac Radiol*. 2004;33(6):396–402. doi:10.1259/dmfr/53801969

6. Panda M, Shankar T, Raut A, Dev S, Kar AK, Hota S. Cone beam computerized tomography evaluation of incisive canal and anterior maxillary bone thickness for placement of immediate implants. *J Indian Prosthodont Soc*. 2018;18(4):356–363. doi:10.4103/jips.jips_167_18

7. Dedhia P, Dedhia S, Dhokar A, Desai A. Nasopalatine duct cyst. *Case Rep Dent*. 2013;2013:869516. doi:10.1155/2013/869516

8. Chung CJ, Nguyen T, Lee JH, Kim KH. Incisive canal remodelling following maximum anterior retraction reduces apical root resorption. *Orthod Craniofac Res*. 2021;24 Suppl 1:59–65. doi:10.1111/ocr.12464

9. Yu JH, Nguyen T, Kim YI, Hwang S, Kim KH, Chung CJ. Morphologic changes of the incisive canal and its proximity to maxillary incisor roots after anterior tooth movement. *Am J Orthod Dentofacial Orthop*. 2022;161(3):396.e1–403.e1. doi:10.1016/j.ajodo.2020.08.022

10. Wilmes B, Ludwig B, Vasudavan S, Nienkemper M, Drescher D. The T-Zone: Median vs. paramedian insertion of palatal mini-implants. *J Clin Orthod*. 2016;50(9):543–551. PMID:27809213.

11. Ghislanzoni LH, Berardinelli F, Ludwig B, Lucchese A. Considerations involved in placing miniscrews near the nasopalatine bundle. *J Clin Orthod*. 2016;50(5):321–328. PMID:27323275.

12. Linjawi AI, Othman MA, Dirham AA, et al. Morphological evaluation of the incisive canal with reference to gender and age: A cone-beam computed tomography study. *Niger J Clin Pract*. 2021;24(11):1596–1601. doi:10.4103/njcp.njcp_40_21

13. Milanovic P, Selakovic D, Vasiljevic M, et al. Morphological characteristics of the nasopalatine canal and the relationship with the anterior maxillary bone – A cone beam computed tomography study. *Diagnostics (Basel)*. 2021;11(5):915. doi:10.3390/diagnostics11050915

14. Rai S, Misra D, Misra A, et al. Significance of morphometric and anatomic variations of nasopalatine canal on cone-beam computed tomography in anterior functional zone – A retrospective study. *Ann Maxillofac Surg*. 2021;11(1):108–114. doi:10.4103/ams.ams_283_20

15. Görürgöz C, Öztaş B. Anatomic characteristics and dimensions of the nasopalatine canal: A radiographic study using cone-beam computed tomography. *Folia Morphol (Warsz)*. 2021;80(4):923–934. doi:10.5603/FM.a2020.0118

16. Bornstein MM, Balsiger R, Sendi P, von Arx T. Morphology of the nasopalatine canal and dental implant surgery: A radiographic analysis of 100 consecutive patients using limited cone-beam computed tomography. *Clin Oral Implants Res*. 2011;22(3):295–301. doi:10.1111/j.1600-0501.2010.02010.x

17. Shrout PE, Fleiss JL. Intraclass correlations: Uses in assessing rater reliability. *Psychol Bull*. 1979;86(2):420–428. doi:10.1037//0033-2909.86.2.420

18. Cicchetti DV, Allison T. A new procedure for assessing reliability of scoring EEG sleep recordings. *Am J EEG Technol*. 1971;11(3):101–109. doi:10.1080/00029238.1971.11080840

19. Koo TK, Li MY. A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J Chiropr Med*. 2016;15(2):155–163. doi:10.1016/j.jcm.2016.02.012

20. Bahşi I, Orhan M, Kervancıoğlu P, Yalçın ED, Aktan AM. Anatomical evaluation of nasopalatine canal on cone beam computed tomography images. *Folia Morphol (Warsz)*. 2019;78(1):153–162. doi:10.5603/FM.a2018.0062

21. Khojastepour L, Haghnegahdar A, Keshtkar M. Morphology and dimensions of nasopalatine canal: A radiographic analysis using cone beam computed tomography. *J Dent (Shiraz)*. 2017;18(4):244–250. PMID:29201966.

22. Thakur AR, Burde K, Guttal K, Naikmasur VG. Anatomy and morphology of the nasopalatine canal using cone-beam computed tomography. *Imaging Sci Dent*. 2013;43(4):273–281. doi:10.5624/isd.2013.43.4.273

23. Gönül Y, Bucak A, Atalay Y, et al. MDCT evaluation of nasopalatine canal morphometry and variations: An analysis of 100 patients. *Diagn Interv Imaging*. 2016;97(11):1165–1172. doi:10.1016/j.diii.2015.11.012

24. Mardinger O, Namani-Sadan N, Chaushu G, Schwartz-Arad D. Morphologic changes of the nasopalatine canal related to dental implantation: A radiologic study in different degrees of absorbed maxillae. *J Periodontol*. 2008;79(9):1659–1662. doi:10.1902/jop.2008.080043

25. Liang X, Jacobs R, Martens W, et al. Macro- and micro-anatomical, histological and computed tomography scan characterization of the nasopalatine canal. *J Clin Periodontol*. 2009;36(7):598–603. doi:10.1111/j.1600-051X.2009.01429.x

Potential barriers to the rational antibiotic use in dental and periodontal practice: A questionnaire-based online survey

Meltem Özdemir Kabalak[1,B–D], Ege Nadir Aytac[1,B,C], Nermin Tarhan[1,A,E,F], Erdem Karabulut[2,C], Hüseyin Gencay Keceli[1,A,E,F]

[1] Department of Periodontology, Faculty of Dentistry, Hacettepe University, Ankara, Turkey

[2] Department of Biostatistics, Faculty of Medicine, Hacettepe University, Ankara, Turkey

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

Address for correspondence
Meltem Özdemir Kabalak
E-mail: dt.meltemozdemir@gmail.com

Abstract

Background. Dentists, through inappropriate antibiotic prescription, may contribute to the global problem of antibiotic resistance (AR).

Objectives. Understanding dentists' antibiotic prescription patterns, source of knowledge, and the driving forces behind their prescription practices may be crucial for the effective implementation of the rational use of antibiotics (RUA) in dentistry.

Material and methods. Active members of the Turkish Dental Association were invited to participate in an electronic survey comprising questions focusing on their role, knowledge and perceptions regarding RUA, the perceived barriers to adapting RUA in daily dental work, and the actual antibiotic prescription practices. The potential impact of age, gender, professional experience, and the mode of dental practice was also evaluated. Dentists' prescription practices for periodontal disease/conditions were evaluated as well.

Results. Based on 1,005 valid responses, there was consensus on the necessity of RUA (99.1%); however, its implementation was low. The main barriers were dentists' own safety concerns (74.4%), strong patients' demands (42.2%) and the fact that prescribing antibiotics became a professional habit (35.8%). Different educational background resulted in clear variances in everyday prescription practices.

Conclusions. The implementation of RUA was not sufficient and the perceived barriers had an impact on daily prescribing habits. Support for dental professionals through the efficient dissemination of evidence-based clinical guidelines and decision-making aids is likely to require additional help from professional organizations in order to actively combat AR.

Keywords: antibiotics, antibiotic resistance, public health dentistry, dental practice pattern, evidence-based dentistry

Introduction

While interventional treatment is becoming more and more successful in controlling dental infections, antibiotics are increasingly being limited to specific therapeutic/prophylactic indications.[1,2] Most healthcare professionals are perfectly aware that the unnecessary use of antibiotics is a global problem; nonetheless, the inappropriate prescription rate is high in both developed and developing countries.[3] Most oral healthcare professionals prescribe antibiotics empirically, either for prophylaxis or for managing infections. However, there is disagreement over whether systemic antibiotics provide therapeutic benefits in all cases.[4] Additionally, the irrational or inappropriate use of antibiotics is still common for caries, gingivitis, pulpitis, and apical inflammation therapy, conditions that could be managed by dental procedures.[5]

Since antibiotic resistance (AR) is a complex, multisectoral and multifactorial problem, it is crucial that the various factors underlying AR be fully evaluated if we are to combat this issue effectively. These factors include: the lack of standardization in prescription; barriers in transferring evidence-based dentistry (EBD) principles to daily practice; and limited dissemination and effective implementation of clinical guidelines (CGs).[6,7] Health professionals' perceptions and attitudes toward AR are also a matter of concern.[7] Considering that dentists are responsible for about 10% of the antibiotics prescribed, dental professionals need to minimize their contribution to AR.[8,9]

In addition to physicians' prescribing behavior, sociodemographic factors (medical education, previous experience, etc.), physicians' attitudes (ignorance, fear, etc.), patient-related factors (allergies, economic and social factors, etc.), healthcare-related factors (time pressure, patient load, etc.), and miscellaneous other factors (e.g., cost-saving), have been suggested to play significant roles with regard to this problem.[2,10] As reported for general medical practice, dentists' decision making concerning antibiotic prescription, selection and timing, as well as treatment duration often varies depending on their experience, knowledge and social factors.[5] Together, these data highlights the role of non-medical factors in the antibiotic prescription practices of healthcare professionals.

When the role of organized dentistry and individual dental professionals in combating AR is considered, the daily prescription practices of dental professionals, the perceived barriers to the effective implementation of the rational use of antibiotics (RUA), the responsibility of organized dentistry, and dental professionals' perceptions and attitudes toward AR and RUA may be of significant importance.[4,8] The analysis of variances in dentists' prescription patterns, and the potential impact of gender, age, years of professional experience, and the mode of practice may also deserve further professional interest. In the present study, our aim was to evaluate AR issues from a dental perspective.

Material and methods

Development of the questionnaire and its validity

A questionnaire was designed to analyze dentists' familiarity with RUA, their perceptions and attitudes toward RUA, the perceived barriers to the effective implementation of RUA, and dentists' antibiotic prescribing habits during their actual practice. The questions were adapted from previous studies and modified in line with the aim of this study.[11] A preparatory survey was carried out to increase the validity and practical applicability of the questionnaire, and to provide further clarification. In the preparatory study, the questionnaire was administered to a group of 30 dentists of different background. The questionnaire was subsequently modified in line with the received feedback.

Structure and content of the questionnaire

The questionnaire basically comprised 2 sections (plus 5 initial questions on demographic data and professional characteristics). The 1st section concerned AR and the principles of RUA, while the 2nd section focused on the implementation of RUA with regard to daily dental practice (taking a prescription for systemic antibiotics for the treatment of periodontal diseases/conditions by dentists as an example). In the 1st section, there were 12 questions concerning dentists' knowledge and perceptions about AR and RUA (one of which was multiple-response), and 2 questions about the medical and non-medical factors affecting dentists' antibiotic prescription (both multiple-response). In the 2nd section, there were a total of 7 questions concerning the benefits, timing, and choice of systemic antibiotics, as well as indications for their use in the treatment of periodontal diseases/conditions. Potential mistakes when prescribing antibiotics for the treatment of periodontal diseases/conditions were also considered (Table 1).

Sample size calculation and conducting the survey

The sample size calculation was based on the total number of the Turkish Dental Association (TDA) members in 2017, which was 17,003. Thus, the required representative sample to reject a null hypothesis at a 0.05 margin of error and a 95% confidence interval (CI) was 376 (the minimum sample size). The TDA conducted the survey electronically among its members between June 9, 2017 and October 1, 2017. All members were asked to participate by sending a link to the survey (recorded as a Google Survey) to their registered e-mail addresses in the source mailing list for TDA.

Table 1. Questionnaire

Section	Questions
A. Demographic data	Gender
	Age
	Years in dental practice
	Professional status/dental education
	Mode of dental practice
B. General questions on antibiotic use in dentistry	1. In dentistry, antibiotics should be used in a rational way.†
	2. In line with the rational use of antibiotics (RUA), dentists should prescribe antibiotics only in necessary cases.†
	3. Dentists prescribe antibiotics only in necessary cases.†
	4. To keep up-to-date with RUA, dentists should be familiar with the most recent knowledge (scientific papers, guidelines, evidence-based data, etc.).†
	5. Dentists are familiar with the most recent knowledge regarding RUA (scientific papers, guidelines, evidence-based data, etc.).†
	6. Dentists' knowledge about RUA is sufficient.†
	7. Where do dentists get their knowledge about RUA from?‡
	8. Information to support dentists with regard to RUA (scientific papers, guidelines, evidence-based data, etc.) is sufficient.†
	9. More emphasis needs to be put on RUA (e.g. in dental schools, dental associations and chambers, specialization associations, at dental congresses, etc.).†
	10. For antibiotics to be used in a rational manner, dentists should inform their patients about RUA.†
	11. Dentists inform their patients about RUA.†
	12. Dentists know the indications, contraindications, mechanisms, side effects, and interactions with other drugs in regard to antibiotics.†
	13. When prescribing antibiotics, which of the following factors do dentists primarily take into consideration?‡
	14. Dentists prescribe antibiotics due to which of the following non-medical factors?‡
C. Use of systemic antibiotics for the treatment of periodontal diseases	15. Dentists keep up-to-date regarding changes in the systemic antibiotic regimen for periodontal treatment.†
	16. In the treatment of periodontal diseases, using only antibiotics, without mechanical debridement, does not provide any benefits.†
	17. What is the ideal timing of using antibiotics in the treatment of periodontal diseases as an adjunct to mechanical debridement? (Please exclude acute periodontal conditions.)#
	18. For which of the following diagnoses are antibiotics needed for treatment?‡
	19. In a medically healthy patient, for which of the following periodontal therapies should antibiotics be used as an adjunct?‡
	20. For which of the following medical conditions are antibiotics used when periodontal treatment is provided?‡
	21. What are the mistakes made when using antibiotics in the treatment of periodontal diseases?‡

† single-response question (answer options: strongly agree; agree; disagree; strongly disagree; or no idea); # single-response question (answer options: before mechanical debridement; during mechanical debridement; after mechanical debridement; no idea; or other); ‡ multiple-response question.

Statistical analysis

Out of the total number of 17,003, 1,005 (5.9%) dentists accepted our invitation to participate (Fig. 1). The survey data was entered into a statistical software package (IBM SPSS Statistics for Windows, v. 24.0; IBM Corp., Armonk, USA).[12] The answer ratios were determined, and statistical analyses were performed. All associations for the variables of gender, age, years in dental practice, and the professional status were analyzed using the χ^2 test. The statistical significance level was set at $p < 0.05$.

Results

Demographic data and professional characteristics

All data of 1,005 dentists participating in the study was included in the statistical analysis phase. In total,
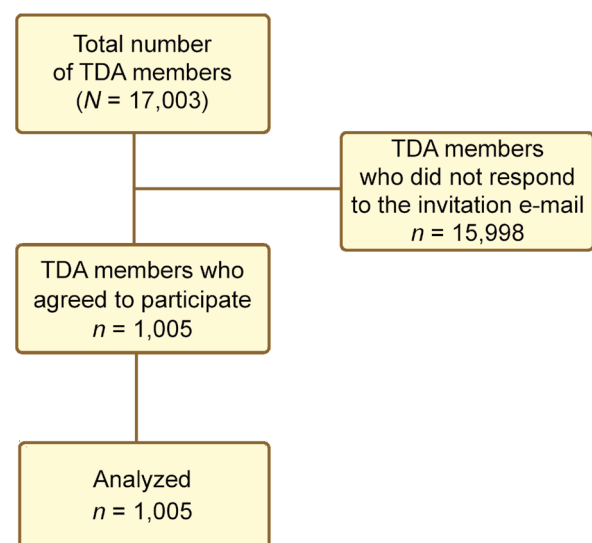
Fig. 1. Flowchart of the study

TDA – Turkish Dental Association.

55.6% of the participants were male and 43.4% of the participants were female, and most (80.3%) were general dental practitioners (GDPs). The dentists' distribution with regard to the total number of years spent in dental practice was as follows: between 1–5 years (25.3%); and more than 25 years (26.3%). In most instances, the dentists' practice mode was a private clinic (47.1%), with a polyclinic/shared practice coming second (27.2%) (Table 2).

Dentists' knowledge and perceptions about RUA

Almost all of the dentists surveyed agreed on RUA (99.1%), and agreed that antibiotics should be prescribed only in case of need (98.9%). However, 69.0% thought that antibiotics were not prescribed only in case of need. According to 71.5%, dentists' knowledge about RUA was insufficient. Only 37.0% of the participants thought that the available scientific information and resources were sufficient to support dentists'

decision making about RUA. Almost all respondents (98.1%) thought that universities and organizations should put more emphasis on RUA (Table 3).

While almost all participants (97.6%) agreed that dentists should inform their patients how to use antibiotics rationally, only 38.9% thought that their colleagues were actually informing patients. According to 68.3% of the dentists, their colleagues did not know enough about the indications, contraindications, mechanisms, side effects, and interactions regarding antibiotics. In general, our data highlighted a significant gap between dentists' beliefs and attitudes and the actual practice regarding AR and RUA (Table 3). Undergraduate education (73.7%) was the primary source of knowledge about RUA, followed by continuing courses (57.2%) and publications (53.2%). Only 23.9% of the participants considered guidelines as the source of dentists' information about RUA (Table 4).

Factors affecting dentists' antibiotic prescription and the potential barriers to adopting RUA

The predominant medical factor considered during prescription was the patient's medical status and/or pregnancy or lactation (76.9%), followed by the patient's history of allergy (75.8%). The dentists listed clinicians' past experience (74.8%), the patient's age (73.7%), disease severity (68.8%), and diagnosis (68.2%) as other main prescription considerations. Evidence regarding antibiotic use was considered only by 17.7% of dentists, while the risk of AR was considered by 38.9% (Table 4). 'Feeling safe' was the non-medical criterion expressed the most (74.4%). The patient's demands (42.2%) and expectations (40.3%) were other non-medical factors considered (Table 4).

Impact of age, gender, professional experience, and the mode of practice

Disagreement with the opinion that dentists complied with RUA increased as years in practice decreased ($p = 0.002$). Additionally, dentists with fewer professional practice years agreed more with the opinion that dentists did not provide adequate information for their patients ($p = 0.008$). While more female respondents were of the opinion that dentists should inform their patients about RUA ($p = 0.003$), more male dentists expressed the opinion that their colleagues prescribed antibiotics only when necessary ($p = 0.029$) (Table 3).

In comparison with specialists, GPDs more believed in the need to inform patients ($p = 0.001$), whereas specialists less believed in the rationality of dentists when prescribing antibiotics and the adequacy of their knowledge about RUA as compared to GPDs ($p < 0.001$). Furthermore, more specialists expressed the opinion that although current scientific resources should be followed by dentists, they were not being followed in practice ($p = 0.001$). In contrast to

Table 2. Demographic data and professional characteristics of the respondents ($N = 1,005$)

Variable		n	%
Gender	unspecified	10	1.0
	male	559^^	55.6
	female	436^	43.4
Age [years]	unspecified	9	0.9
	21–30	287^^	28.6
	31–40	262	26.1
	41–50	205	20.4
	51–60	143	14.2
	>60	99^	9.9
Years in practice	unspecified	9	0.9
	1–5	254	25.3
	6–10	168	16.7
	11–15	109	10.8
	16–20	113	11.2
	21–25	88^^	8.8
	>25	26^	26.3
Professional status	GPD	807^^	80.3
	specialist	198^	19.7
Mode of practice	unspecified	12	1.2
	private practice	473^^	47.1
	university	73	7.3
	public hospital	28	2.8
	public health center	76	7.6
	private hospital	61	6.1
	polyclinic/shared practice	273	27.2
	other	9^	0.9

GDP – general dental practitioner; ^^ most common answer; ^ least common answer.

Table 3. Dentists' knowledge and perceptions about the rational use of antibiotics (RUA), and the effect of gender, age, years in practice, the professional status, and the mode of practice

Questions	Answer options	n	%	p-value				
				gender	age	years in dental practice	professional status/dental education	mode of dental practice
In dentistry, antibiotics should be used in a rational way	strongly agree	913^^	90.8	0.413	0.040*	0.023*	0.253	0.961
	agree	83	8.3					
	disagree	2	0.2					
	strongly disagree	1^	0.1					
	no idea	6	0.6					
In line with RUA, dentists should prescribe antibiotics only in necessary cases	strongly agree	863^^	85.9	0.986	0.216	0.716	0.029*	0.999
	agree	131	13.0					
	disagree	2^	0.2					
	strongly disagree	2^	0.2					
	no idea	7	0.7					
Dentists prescribe antibiotics only in necessary cases	strongly agree	127	12.6	0.029*	<0.001*	<0.001*	<0.001*	0.307
	agree	159	15.8					
	disagree	532^^	52.9					
	strongly disagree	162	16.1					
	no idea	25^	2.5					
To keep up-to-date with RUA, dentists should be familiar with the most recent knowledge	strongly agree	692^^	68.9	0.015*	0.542	0.764	<0.001*	0.508
	agree	293	29.2					
	disagree	5	0.5					
	strongly disagree	1^	0.1					
	no idea	14	1.4					
Dentists are familiar with the most recent knowledge regarding RUA	strongly agree	72	7.2	0.007*	0.002*	0.003*	<0.001*	0.747
	agree	143	14.2					
	disagree	593^^	59.0					
	strongly disagree	130	12.9					
	no idea	67^	6.7					
Dentists' knowledge about RUA is sufficient	strongly agree	29^	2.9	0.041*	0.006*	0.002*	<0.001*	0.879
	agree	213	21.2					
	disagree	601^^	59.8					
	strongly disagree	118	11.7					
	no idea	44	4.4					
Information to support dentists with regard to RUA is sufficient	strongly agree	43^	4.3	0.119	0.448	0.380	<0.001*	0.002*
	agree	329	32.7					
	disagree	502^^	50.0					
	strongly disagree	80	8.0					
	no idea	51	5.1					
More emphasis needs to be put on RUA	strongly agree	728^^	72.4	0.096	0.681	0.858	0.449	0.999
	agree	258	25.7					
	disagree	6	0.6					
	strongly disagree	0^	0.0					
	no idea	13	1.3					
For antibiotics to be used in a rational manner, dentists should inform their patients about RUA	strongly agree	699^^	69.6	0.003*	0.686	0.752	0.042*	0.894
	agree	281	28.0					
	disagree	11	1.1					
	strongly disagree	2^	0.2					
	no idea	12	1.2					
Dentists inform their patients about RUA	strongly agree	63	6.3	0.164	0.013*	0.008*	0.001*	0.194
	agree	328	32.6					
	disagree	505^^	50.2					
	strongly disagree	58	5.8					
	no idea	51^	5.1					
Dentists know the indications, contraindications, mechanisms, side effects, and interactions with other drugs in regard to antibiotics	strongly agree	29^	2.9	0.908	0.205	0.143	<0.001*	0.027*
	agree	243	24.2					
	disagree	578^^	57.5					
	strongly disagree	109	10.8					
	no idea	46	4.6					

^^ most common answer; ^ least common answer; * statistically significant (χ^2 test).

Table 4. Dentists' sources of knowledge about the rational use of antibiotics (RUA) and the factors affecting dentists' antibiotic prescription (multiple-response questions)

Questions	Answer options	n	%
Where do dentists get their knowledge about RUA from?	undergraduate education	741^^	73.7
	postgraduate/specialization education	326	32.4
	continuing courses	575	57.2
	publications	535	53.2
	the Internet	358	35.6
	colleagues	494	49.2
	medical representatives	334	33.2
	media	86	8.6
	professional organizations	172	17.1
	guidelines	240	23.9
	scientific meetings	452	45.0
	other	14	1.4
Which factors are primarily taken into consideration during prescription?	risk of AR	391	38.9
	cost of antibiotics	199	19.8
	individual past experience	752	74.8
	date of the patient's last antibiotic use	407	40.5
	frequency of the patient's antibiotic use	350	34.8
	diagnosis	685	68.2
	disease severity	691	68.8
	efficacy of the prescribed antibiotic	545	54.2
	disease symptoms	525	52.2
	patient's genetic status	97	9.7
	patient's systemic status and/or pregnancy or lactation	773^^	76.9
	age	741	73.7
	colleagues	199	19.8
	symptoms of an accompanying systemic infection	632	62.9
	evidence regarding antibiotic use	178	17.7
	gender	45	4.5
	patient's history of allergy	762	75.8
	patient's nutritional habits	60	6.0
	BMI	252	25.1
	other drugs used	483	48.1
	other	8	0.8
Dentists prescribe antibiotics due to which of the following non-medical factors?	patient's expectations	405	40.3
	patient's demands	424	42.2
	patient's positive attitude	326	32.4
	insufficient EBD	60	6.0
	safety feeling after prescription	748^^	74.4
	professional habit	360	35.8
	other	12	1.2

AR – antibiotic resistance; BMI – body mass index; EBD – evidence-based dentistry; ^^ most common answer.

GPDs, most specialists expressed the opinion that the available scientific sources were sufficient ($p < 0.001$). However, more dentists working at universities were of the opinion that the available scientific resources were sufficient as compared to dentists working in private clinics, polyclinics or hospitals ($p = 0.002$) (Table 3).

Prescription of systemic antibiotics for the treatment of periodontal diseases/conditions

Periodontal diseases/conditions associated with systemic signs and symptoms (83.9%), periodontal abscess (58.3%) and necrotizing ulcerative periodontitis (52.4%) were the most frequent indications for antibiotic prescription. In otherwise healthy individuals, the most frequent cases in which antibiotics were prescribed were cases of regenerative treatment (69.3%) and periodontal surgery (63.5%) (Table 5). Although almost all of the participating dentists believed that systemic antibiotics without mechanical debridement were not beneficial, there were significant differences in the ideal timing for the prescription of antibiotics as an adjunct to mechanical debridement (before: 21.3%; during: 33.9%; after: 35.8%). In addition, age ($p < 0.001$), years in dental practice ($p < 0.001$), the professional status ($p = 0.008$),

Table 5. Periodontal practice-specific questions about decisions on the use of systemic antibiotics (multiple-response questions)

Questions	Answer options	n	%
For which of the following diagnoses, are antibiotics needed for treatment?	chronic gingivitis	27	2.7
	chronic periodontitis	73	7.3
	necrotizing ulcerative gingivitis	482	48.0
	aggressive periodontitis	498	49.6
	periodontal abscess	586	58.3
	refractory periodontitis	94	9.4
	mucogingival problems	25	2.5
	gingival abscess	267	26.6
	necrotizing ulcerative periodontitis	527	52.4
	desquamative gingivitis	42	4.2
	gingival hyperplasia	22	2.2
	recession with hypersensitivity	0^	0.0
	herpes lesions	12	1.2
	recurrent periodontitis	107	10.6
	non-specific aphthous lesions	9	0.9
	non-specific ulcerations	14	1.4
	fungal infections	67	6.7
	perio-endo lesions	237	23.6
	periodontal conditions with systemic symptoms	843^^	83.9
	other	6	0.6
In a medically healthy patient, for which of the following periodontal therapies should antibiotics be used as an adjunct?	hand scaling	12^	1.2
	ultrasonic scaling	16	1.6
	subgingival curettage	136	13.5
	periodontal surgery	638	63.5
	periodontal abscess treatment	625	62.2
	regenerative treatment	696^^	69.3
	other	13	1.3
For which of the following medical conditions are antibiotics used when periodontal treatment is provided?	controlled diabetes	54	5.4
	uncontrolled diabetes	578	57.5
	radiotherapy	395	39.3
	chemotherapy	422	42.0
	immune disorders	613	61.0
	osteoporosis	36	3.6
	mitral valve problems	907^^	90.2
	bypass surgery	386	38.4
	joint prosthesis	488	48.6
	corticosteroid use	213	21.2
	bisphosphonate use	219	21.8
	HIV/AIDS	265	26.4
	rheumatoid arthritis	405	40.3
	hyperlipidemia	7^	0.7
	pregnancy	8	0.8
	bleeding disorders	27	2.7
	immunosuppressive drug use	540	53.7
	other	9	0.9

HIV – human immunodeficiency virus ; AIDS – acquired immunodeficiency syndrome; ^^ most common answer; ^ least common answer.

and the mode of practice ($p < 0.001$) all had a significant impact on the preferred timing for antibiotic treatment. Most of the participants (62.5%) felt that dentists did not keep up-to date-with the current regimens for antibiotic prescription in the treatment of periodontal diseases/conditions. Again, age ($p < 0.001$), years in dental practice ($p < 0.001$), the professional status ($p < 0.001$), and the mode of practice ($p < 0.001$) all had an impact on the responses (Table 6).

Prophylaxis seemed to be a major indication for systemically compromised cases undergoing dental treatment (e.g., mitral valve prolapse, immune system dysfunction, etc.). The incorrect indication (64.6%), the incorrect choice of antibiotics (62.1%) and the early cessation of antibiotic treatment (61.0%) were listed as the major mistakes made when prescribing systemic antibiotics in the treatment of periodontal diseases/conditions (Table 7).

Table 6. Periodontal practice-specific questions on systemic antibiotic use and preferences, and the effect of gender, age, years in practice, the professional status, and the mode of practice

Questions	Answer options	n	%	p-value				
				gender	age	years in dental practice	professional status/dental education	mode of dental practice
Dentists keep up-to-date regarding changes in the systemic antibiotic regimen for periodontal treatment	strongly agree	43^	4.3	0.312	<0.001*	<0.001*	<0.001*	<0.001*
	agree	253	25.2					
	disagree	552^^	54.9					
	strongly disagree	76	7.6					
	no idea	81	8.1					
In the treatment of periodontal diseases, using only antibiotics, without mechanical debridement, does not provide any benefits	strongly agree	622^^	61.9	0.028*	0.015*	0.090	0.113	0.476
	agree	304	30.2					
	disagree	44	4.4					
	strongly disagree	10	1.0					
	no idea	25^	2.5					
What is the ideal timing of using antibiotics in the treatment of periodontal diseases as an adjunct to mechanical debridement (MD)? (Please exclude acute periodontal conditions.)	before MD	214	21.3	0.153	<0.001*	<0.001*	0.008*	<0.001*
	during MD	341	33,9					
	after MD	360^^	35.8					
	no idea	90^	9.0					

^^ most common answer; ^ least common answer; * statistically significant (χ^2 test).

Table 7. Mistakes made in the use of systemic antibiotics when treating periodontal diseases (multiple-respone question)

What are the mistakes made when using antibiotics in the treatment of periodontal diseases?	n	%
Incorrect indication	649^^	64.6
Incorrect timing	522	51.9
Incorrect antibiotic selection	624	62.1
Incorrect dose/application mode	400	39.8
Discontinuing treatment	613	61.0
Not considering drug interactions	366	36.4
Insufficient disease knowledge	526	52.3
Insufficient treatment. knowledge	486	48.4
Habit of irrational use	547	54.4
Misleading to the patient's expectations	365	36.3
Insufficient evidence	132^	13.1
Limited EBD awareness	347	34.5
Prescription of the same antibiotic due to the limited knowledge about antibiotics	583	58.0

^^ most common answer; ^ least common answer.

Discussion

Since the evaluation of the actual antibiotic prescription practices of dentists in their daily dental work was the main aim of the present study, the 2nd section of the questionnaire was devoted to periodontal practice and dentists' prescription patterns for the treatment of periodontal diseases/conditions. Although academic knowledge may not always be translated to dental practice, the up-to-date knowledge of RUA and appropriate antibiotic prescription practices among health professionals are still required.[5,13] The suboptimal knowledge of dental professionals in the field of RUA and clear variations in antibiotic prescribing practices among dentists have been frequently mentioned in earlier studies.[1,5,14–17] In the present study, most of the dentist respondents highlighted the suboptimal knowledge of dentists regarding the principles of RUA (71.5%) and their inability to keep up-to-date in this field (71.9%). Although all dentists acknowledged AR as a serious health problem, they doubted if RUA was effectively implemented into daily dental work. Interestingly, suboptimal knowledge was addressed more as years in practice decreased (p = 0.002). In line with previous studies,[13,14] the failure of dentists to keep up-to-date with best practices for prescribing systemic antibiotics in the treatment of periodontal diseases/conditions (62.5%) was again confirmed. Furthermore, there were clear differences in the appropriate timing of antibiotics reported and in appropriate periodontal antibiotic indications. Again, these results support previous studies suggesting the lack of standardized prescription procedure for dentists in daily practice.[14,15,18]

For the majority of the participating dentists, the need for effective knowledge transfer to enable dentists to make decisions using the most recent, reliable and evidence-based resources was acknowledged, which again was in line with previous studies.[7,14] In this respect, well-conducted stewardship courses and training programs (e.g., continuing education courses, dental congresses, etc.) addressing the needs of the whole dental team have been broadly suggested.[17,19] In the present study, only 37.0% of the participants believed that the scientific information and resources concerning RUA readily available were

sufficient to support dentists, while almost all (98.1%) thought that universities and organizations had to put more emphasis on RUA.

Pre-appraised evidence (e.g., systematic reviews, CGs, etc.) may help dentists in making evidence-based decisions,[16,17] and CGs such as "Drug prescribing for dentistry",[20] the evidence-based clinical practice guideline on the nonsurgical treatment of chronic periodontitis[21] and "Prevention and management of dental caries in children"[22] may be important for the effective implementation of RUA. These documents recommend antibiotics only for cases when they are strictly indicated (e.g., systemic involvement or in conjunction with mechanical dental/periodontal treatment).[23] However, in numerous countries, CGs do not seem to be systematically disseminated or effectively implemented.[24] A cross-sectional study reported that only 19% of antibiotic prescription by UK dentists was compliant with CGs;[18] many practitioners were either not aware of CGs or considered them as a reducer of their professional autonomy, and only about 50% of the practitioners found CGs helpful in daily clinical decision-making.[7] In the present study, while undergraduate education (73.7%) and continuing educational courses (57.2%) were reported as the major sources of information, only 23.9% of the dentists benefited from CGs about RUA. Since almost all participants in the present study supported the need for more emphasis to be placed on RUA by dental organizations, additional efforts from these organizations are required to develop and disseminate CGs that would increase knowledge regarding AR and RUA. Moreover, dental organizations should also provide support for the whole dental team through other decision-making support materials (e.g., publications, courses, audits, patient education materials, awareness campaigns, etc.).

As the main reasons for the growth of AR comprise patients not completing their course of treatment, self-prescription (including the use of leftover antibiotics), and poor hygiene and sanitation habits, the education of dental patients may be paramount in combating AR.[25] A qualitative study conducted amongst adolescents revealed that antibiotics were perceived by patients on the same level as analgesics, as a cure-all for any illness.[26] Thus, health professionals are expected to inform their patients about their role, responsibility about AR and its significant consequences.[4] In the present study, despite the almost universal (97.6%) belief that dentists should provide information to their patients concerning AR and RUA, our respondents felt that this was ignored in daily dental practice (56.0%). All health professionals need to reconsider their role and responsibility in combating the global problem of AR. This can be achieved by effectively communicating to their patients updated information about RUA, AR and the negative outcomes of antibiotic misuse, and by helping them reach the necessary educational resources.[27] Additionally, this may also help

dentists manage the demands of dental patients for antibiotics, which is an important driving force for inappropriate and unnecessary antibiotic prescription by the dentists.[24] This problem was also mentioned in the present study.

The observed heterogeneity in dentists' antibiotic prescription practices and the driving forces behind this heterogeneity, together with the potential barriers to effective implementation of RUA, are of particular importance for developing strategies to achieve compliance with RUA.[6,7,16] Other studies have confirmed this fact, with up to 80% of antibiotic prescriptions by UK dentists found to be either unnecessary or inappropriate.[9] Similarly, Fleming-Dutra et al. reported that a 30% reduction in prescriptions was achievable.[28] In the present study, 69.0% of the dentist respondents believed that unnecessary antibiotics were prescribed by dentists. The 2 major non-medical driving forces for prescriptions were listed as the practitioners' own safety (74.4%), and patient-related factors, including the patient's demands (42.2%) and expectations (40.3%). Together, these findings emphasize the importance of enhancing the quality of prescribing in dentistry.[29]

Broad-spectrum antibiotics are generally prescribed by dentists empirically for prophylaxis or to manage oral/dental infections.[15,25] Amoxicillin (alone or in combination with clavulanic acid), metronidazole, clindamycin, and azithromycin are commonly used systemic antibiotics in dental practice.[30–32] However, there are conflicting reports on whether systemic antibiotics provide a therapeutic benefit in all cases. Alternative approaches, including the use of drug delivery systems that keep the local antimicrobial concentration in the application area at a high level, reduce the need for systemic antibiotic use in dentistry, and they have a decreased associated risk of promoting AR.[33] Numerous local drug delivery systems have been successfully trialed, including fibers, gels, membranes, microparticles, nanoparticles, and liposomes, as well as novel carrier forms developed for this purpose.[2,34] Constant levels of antibiotics, including tetracycline, doxycycline, minocycline, metronidazole, etc., can be maintained using these systems.[2,34–39] It should also be stated that the recommended interventions (e.g., root canal therapy, scaling and root planing) are effective, and that the antibiotic need is very limited in practice.[24,29,40]

Although the medical status, a history of allergy and disease severity were among the most frequently considered factors in the present study, a unanimous set of criteria for antibiotic prescription in terms of medical/dental reasoning was not observed. It can be concluded that what should be in the focus is the individual risk assessment in decision making for prescription.[41] Dentists are expected to make judicious use of antimicrobials and prescribe the correct drug at the standard dosage in the appropriate regimen, and only when a real need is evident.[24,29,42] However, it is known that health professionals'

decisions are affected by a wide array of factors, including educational and training background, and differences in local circumstances. Their attitudes and perceptions are also important. At this point, the perceived barriers to effective implementation of RUA expressed by dentists are naturally of utmost importance. Dentists report the lack of time, education and accessible CGs as the major barriers to adapting EBD to daily practice.[7] In future endeavors, the multifactorial and multisectoral nature of AR should be considered, and the need for partnerships, collaborations and innovative approaches involving different stakeholders should be taken into account as well. Together, these considerations can initiate cooperation between different parties, including organized dentistry.[16] Although there are encouraging steps,[8] AR remains a global problem despite all our efforts. This needs to be acknowledged by the dental profession (dental organizations, individual dentists, and other dental team members), and effective measures should be taken to combat AR.

Limitations

An inherent limitation of our study is that it was based on a survey that included questions evaluating individual perceptions. However, the high number of dentist participants increases the strength of our study. Another limitation is that the study was based on a single-country survey, whilst antibiotic usage habits may differ in each country. Nevertheless, the information obtained here should help to generally address barriers to RUA in dentistry.

Conclusions

Dental health professionals have several important roles to play in combating AR, including preventing the unnecessary prescription and/or misuse of antibiotics, informing patients about AR and RUA, keeping up-to-date with advances in this field, and making evidence-based decisions using the most current and reliable data. Considering problems with prescription practices, professional attitudes toward RUA, the level and extent of dentists' knowledge, and the perceived barriers, practitioners may need professional guidance to effectively implement RUA into daily work. Furthermore, based on the impact of gender, age, and the mode and years of practice on the antibiotic prescription patterns of dentists, trends in AR and RUA, and the prescription patterns of dentists may need additional monitoring.

Ethics approval and consent to participate

The study protocol was approved by the ethics committee at the Hacettepe University, Ankara, Turkey, in accordance with the Declaration of Helsinki 1975, as revised in 2008 (No. GO 17/490-10).

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

Meltem Özdemir Kabalak   https://orcid.org/0000-0002-5637-3362
Ege Nadir Aytac   https://orcid.org/0000-0002-6790-7411
Nermin Tarhan   https://orcid.org/0000-0003-3218-4409
Erdem Karabulut   https://orcid.org/0000-0002-7811-8215
Hüseyin Gencay Keceli   https://orcid.org/0000-0001-6695-2133

References

1. Koyuncuoglu CZ, Aydin M, Kirmizi NI, et al. Rational use of medicine in dentistry: Do dentists prescribe antibiotics in appropriate indications? *Eur J Clin Pharmacol*. 2017;73(8):1027–1032. doi:10.1007/s00228-017-2258-7
2. Buonavoglia A, Leone P, Solimando AG, et al. Antibiotics or no antibiotics, that is the question: An update on efficient and effective use of antibiotics in dental practice. *Antibiotics (Basel)*. 2021;10(5):550. doi:10.3390/antibiotics10050550
3. Gowri S, Mehta D, Kannan S. Antibiotic use in dentistry: A cross-sectional survey from a developing country. *J Orofac Sci*. 2015;7(2):90–94. doi:10.4103/0975-8844.164310
4. Ramasamy A. A review of use of antibiotics in dentistry and recommendations for rational antibiotic usage by dentists. *Int Arab J Antimicrob Agents*. 2014;4(2):1–15 doi:10.3823/748
5. Marra F, George D, Chong M, Sutherland S, Patrick DM. Antibiotic prescribing by dentists has increased: Why? *J Am Dent Assoc*. 2016;147(5):320–327. doi:10.1016/j.adaj.2015.12.014
6. Carrilho E, Dianiskova S, Guncu GN, Nemli SK, Melo P, Yamalik N. Practical implementation of evidence-based dentistry into daily dental practice through a short time dependent searching method. *J Evid Based Dent Pract*. 2016;16(1):7–18. doi:10.1016/j.jebdp.2015.12.001
7. Yamalik N, Nemli SK, Carrilho E, et al. Implementation of evidence-based dentistry into practice: Analysis of awareness, perceptions and attitudes of dentists in the World Dental Federation – European Regional Organization zone. *Int Dent J*. 2015;65(3):127–145. doi:10.1111/idj.12160
8. Thompson W, Williams D, Pulcini C, Sanderson S, Calfon P, Verma M. World Dental Federation white paper: The essential role of the dental team in reducing antibiotic resistance. https://www.fdiworlddental.org/sites/default/files/2020-11/abr_white_paper_english.pdf. Accessed November 25, 2022.
9. Thompson W, Williams D, Pulcini C, Sanderson S, Calfon P, Verma M. Tackling antibiotic resistance: Why dentistry matters. *Int Dent J*. 2021;71(6):450–453. doi:10.1016/j.identj.2020.12.023
10. Rodrigues AT, Roque F, Falcão A, Figueiras A, Herdeiro MT. Understanding physician antibiotic prescribing behaviour: A systematic review of qualitative studies. *Int J Antimicrob Agents*. 2013;41(3):203–212. doi:10.1016/j.ijantimicag.2012.09.003
11. Cope AL, Francis NA, Wood F, Chestnutt IG. Antibiotic prescribing in UK general dental practice: A cross-sectional study. *Community Dent Oral Epidemiol* .2016;44(2):145–153. doi:10.1111/cdoe.12199
12. Hinton PR, McMurray I, Brownlow C. *SPSS Explained*. 2nd ed. London, UK: Routledge; 2014. doi:10.4324/9781315797298
13. Ryalat S, Hassona Y, Al-Shayyab M, Abo-Ghosh M, Sawair F. Dentists' knowledge and practice regarding prevention of infective endocarditis. *Eur J Dent*. 2016;10(4):480–485. doi:10.4103/1305-7456.195158
14. Punj A, Shenoy SB, Thomas B, Ramesh A. Knowledge awareness and prescription practice of antibiotics among private dental practitioners in Mangalore. *J Educ Ethics Dent*. 2016;6(2):72. doi:10.4103/jeed.jeed_1_17

15. Dekker AR, Verheij TJ, Broekhuizen BD, et al. Effectiveness of general practitioner online training and an information booklet for parents on antibiotic prescribing for children with respiratory tract infection in primary care: A cluster randomized controlled trial. *J Antimicrob Chemother*. 2018;73(5):1416–1422. doi:10.1093/jac/dkx542

16. Mölstad S, Löfmark S, Carlin K, et al. Lessons learnt during 20 years of the Swedish strategic programme against antibiotic resistance. *Bull World Health Organ*. 2017;95(11):764–773. doi:10.2471/BLT.16.184374

17. World Health Organization (WHO). Antimicrobial resistance and primary health care. 2018. https://apps.who.int/iris/bitstream/handle/10665/326454/WHO-HIS-SDS-2018.56-eng.pdf?sequence=1&isAllowed=y. Accessed November 25, 2022.

18. Durkin MJ, Hsueh K, Sallah YH, et al. An evaluation of dental antibiotic prescribing practices in the United States. *J Am Dent Assoc*. 2017;148(12):878–886.e1. doi:10.1016/j.adaj.2017.07.019

19. Cox JA, Vlieghe E, Mendelson M, et al. Antibiotic stewardship in low- and middle-income countries: The same but different? *Clin Microbiol Infect*. 2017;23(11):812–818. doi:10.1016/j.cmi.2017.07.010

20. Scottish Dental Clinical Effectiveness Programme. Drug prescribing for dentistry. https://www.sdcep.org.uk/published-guidance/drug-prescribing. Accessed November 25, 2022.

21. Smiley CJ, Tracy SL, Abt E, et al. Evidence-based clinical practice guideline on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. *J Am Dent Assoc*. 2015;146(7):525–535. doi:10.1016/j.adaj.2015.01.026

22. Scottish Dental Clinical Effectiveness Programme. Prevention and management of dental caries in children. https://www.sdcep.org.uk/published-guidance/caries-in-children. Accessed January 15, 2023.

23. Guncu GN, Nemli SK, Carrilho E, et al. Clinical guidelines and implementation into daily dental practice. *Acta Med Port*. 2018;31(1):12–21. doi:10.20344/amp.8942

24. Stein K, Farmer J, Singhal S, Marra F, Sutherland S, Quiñonez C. The use and misuse of antibiotics in dentistry: A scoping review. *J Am Dent Assoc*. 2018;149(10):869–884.e5. doi:10.1016/j.adaj.2018.05.034

25. Review on Antimicrobial Resistance (AMR). Tackling drug-resistant infections globally: Final report and recommendations. 2016. https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf. Accessed January 15, 2023.

26. Hawking MK, Lecky DM, Lundgren PT, et al. Attitudes and behaviours of adolescents towards antibiotics and self-care for respiratory tract infections: A qualitative study. *BMJ Open*. 2017;7(5):e015308. doi:10.1136/bmjopen-2016-015308

27. Gualano MR, Gili R, Scaioli G, Bert F, Siliquini R. General population's knowledge and attitudes about antibiotics: A systematic review and meta-analysis. *Pharmacoepidemiol Drug Saf*. 2015;24(1):2–10. doi:10.1002/pds.3716

28. Fleming-Dutra KE, Hersh AL, Shapiro DJ, et al. Prevalence of inappropriate antibiotic prescriptions among US ambulatory care visits, 2010–2011. *JAMA*. 2016;315(17):1864–1873. doi:10.1001/jama.2016.4151

29. Oberoi SS, Dhingra C, Sharma G, Sardana D. Antibiotics in dental practice: How justified are we. *Int Dent J*. 2015;65(1):4–10. doi:10.1111/idj.12146

30. Thornhill MH, Dayer MJ, Durkin MJ, Lockhart PB, Baddour LM. Oral antibiotic prescribing by NHS dentists in England 2010–2017. *Br Dent J*. 2019;227(12):1044–1050. doi:10.1038/s41415-019-1002-3

31. Thompson W, Teoh L, Hubbard CC, et al. Patterns of dental antibiotic prescribing in 2017: Australia, England, United States, and British Columbia (Canada). *Infect Control Hosp Epidemiol*. 2022;43(2):191–198. doi:10.1017/ice.2021.87

32. Agossa K, Sy K, Mainville T, et al. Antibiotic use in periodontal therapy among French dentists and factors which influence prescribing practices. *Antibiotics (Basel)*. 2021;10(3):303. doi:10.3390/antibiotics10030303

33. Berberich C, Sanz-Ruiz P. Risk assessment of antibiotic resistance development by antibiotic-loaded bone cements: Is it a clinical concern? *EFORT Open Rev*. 2019;4(10):576–584. doi:10.1302/2058-5241.4.180104

34. Sholapurkar A, Sharma D, Glass B, Miller C, Nimmo A, Jennings E. Professionally delivered local antimicrobials in the treatment of patients with periodontitis – a narrative review. *Dent J*. 2020;9(1):2. doi:10.3390/dj9010002

35. Paradowska-Stolarz A, Wieckiewicz M, Owczarek A, Wezgowiec J. Natural polymers for the maintenance of oral health: Review of recent advances and perspectives. *Int J Mol Sci*. 2021;22(19):10337. doi:10.3390/ijms221910337

36. Kubasiewicz-Ross P, Fleischer M, Pitułaj A, et al. Evaluation of the three methods of bacterial decontamination on implants with three different surfaces. *Adv Clin Exp Med*. 2020;29(2):177–182. doi:10.17219/acem/112606

37. Fernandes T, Bhavsar C, Sawarkar S, D'souza A. Current and novel approaches for control of dental biofilm. *Int J Pharm*. 2018;536(1):199–210. doi:10.1016/j.ijpharm.2017.11.019

38. Kida D, Karolewicz B, Junka A, et al. Metronidazole-loaded porous matrices for local periodontitis treatment: In vitro evaluation and in vivo pilot study. *Appl Sci*. 2019;9(21):4545. doi:10.3390/app9214545

39. Donmezer CM, Bilginaylar K. Comparison of the postoperative effects of local antibiotic versus systemic antibiotic with the use of platelet-rich fibrin on impacted mandibular third molar surgery: A randomized split-mouth study. *Biomed Res Int*. 2021;2021:3040661. doi:10.1155/2021/3040661

40. Teoh L, Stewart K, Marino R, McCullough M. Antibiotic resistance and relevance to general dental practice in Australia. *Aust Dent J*. 2018;63(4):414–421. doi:10.1111/adj.12643

41. Cahill TJ, Dayer M, Prendergast B, Thornhill M. Do patients at risk of infective endocarditis need antibiotics before dental procedures? *BMJ*. 2017;358:j3942. doi:10.1136/bmj.j3942

42. Fluent MT, Jacobsen PL, Hicks LA; OSAP, the Safest Dental Visit. Considerations for responsible antibiotic use in dentistry. *J Am Dent Assoc*. 2016;147(8):683–686. doi:10.1016/j.adaj.2016.04.017

Inverted amino acids reduce the adhesion and biofilm biomass of early oral colonizers

Gayan Kanchana Wijesinghe[1,2,A–D,F], Malshani Nissanka[3,D–F], Flávia Camila Maia[2,A–C,F], Thaís Rossini de Oliveira[2,A–C,F], José Francisco Höfling[2,A,C,E,F]

[1] Bristol Dental School, University of Bristol, UK

[2] Piracicaba Dental School, State University of Campinas, Brazil

[3] Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka

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

Address for correspondence
Gayan Kanchana Wijesinghe
E-mail: gayan_k.wijesinghe@bristol.ac.uk

Abstract

Background. Early colonizers adhere to the dental surface and facilitate the initial adhesion of secondary colonizers to form oral biofilms, which may cause oral infections.

Objectives. This study aimed to determine the antimicrobial, anti-adhesion and antibiofilm potency of inverted amino acids on early colonizer streptococci and their mixed species.

Material and methods. The following test strains were used: *Streptococcus gordonii* (American Type Culture Collection (ATCC) 35105); *Streptococcus mitis* (ATCC 49456); *Streptococcus oralis* (ATCC 10557); *Streptococcus salivarius* (ATCC 7073); and *Streptococcus sanguinis* (ATCC BAA-1455). The concentration-dependent antimicrobial potency of d-alanine (d-ala), d-arginine (d-arg), d-leucine (d-leu), d-methionine (d-met), and d-tryptophan (d-try) was determined using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method with AlamarBlue modification. The adhesion of primary colonizers in the presence of 25-mM d-amino acids (dAAs) was assessed using the colony forming unit (CFU) assay. The CFU assay was conducted on 24-h flow cell bacterial biofilm models after exposure to 25-mM inverted dAAs.

Results. No minimum inhibitory concentration (MIC) point was detected at any concentration tested. The minimum bactericidal concentration (MBC) point was not observed. The adhesion of *S. mitis*, *S. oralis* and mixed species was reduced by all tested dAAs. No adverse effects were observed on *S. gordonii* with any of the tested dAAs. The biofilm biomass of test strains under flow conditions was significantly reduced after a 5-min exposure to all tested dAAs at 25-mM concentration.

Conclusions. D-amino acids did not inhibit bacterial growth and did not show bactericidal or bacteriostatic effects on test strains at any concentration tested (ranging from 6.25 mM to 100 mM). However, dAAs effectively inhibit the adhesion of early colonizers, thereby preventing the formation of oral biofilm.

Keywords: biomass, methionine, biofilms, anti-infective agents

Introduction

The human oral cavity, including mucosal and tooth surfaces, provides a natural habitat for various types of microorganisms. It represents one of the body surfaces with a huge population of diverse microorganisms.[1] In the oral cavity, these microorganisms attach to the oral surfaces and form three-dimensional structures, biofilms. These are surface-attached microbial communities that are enclosed in a matrix of extracellular material derived from the cells themselves and from the environment.[2] The attached surface could be either biotic or abiotic.[3] Oral biofilm formation is a complex and dynamic process. First, pellicles are formed on oral surfaces by adsorbing salivary proteins and glycoproteins. The surface conditioning film is known as the acquired enamel pellicle. The major components of a pellicle are salivary glycoproteins, phosphoproteins, lipids, and other host molecules. Oral bacteria generally possess more than one type of adhesion molecule, including integrins, cadherins and selectins, on their surface. They can participate in multiple interactions with host surface molecules and similar receptors on other microorganisms. The dental biofilm pioneer species modify local ecological conditions and promote further colonization by other species that may lead to diseases, such as caries and periodontal diseases.[1]

In the first 6 h of formation, the dental biofilm microbiota is mainly composed of early colonizers, including *Streptococcus* spp. (*Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus salivarius*, *Streptococcus gordonii*, and *Streptococcus sanguinis*).[4,5] These species possess adhesins and other bacterial surface ligands with affinity to host dental-pellicle molecules.[1] Biofilms are significantly different compared to their planktonic counterparts.[6,7] The community lifestyle provides a multitude of benefits to the participating organisms.[8] These include a broader habitat range for growth produced by the metabolism of early colonizers. Early colonizers alter the local environment, creating an optimal environment for the attachment and growth of late colonizers.[9]

The oral microbiota plays an important role in both oral and systemic health.[10] It contributes to host health by maintaining homeostasis within the oral cavity, resisting pathogens and modulating the immune system.[10,11] Furthermore, oral microbiota thwarts disease progression by preventing the adherence of pathogens onto specific surfaces, degrading the pathogen's virulence factors, and hindering the pathogen's ability to multiply.[11] When the sensitive ecosystem within the oral cavity gets out of balance, either by overload or a weak immune system, the oral microbiota shows pathogenic potential.[10]

Although early colonizers are not identified as pathogens, they are the early contributors to the formation of pathogenic polymicrobial oral biofilms. Therefore, the most effective strategy of oral biofilm control is to prevent early colonization. This study is aimed at identifying an effective treatment option against early colonizers.

Biofilms exhibit greater resistance to antimicrobials compared to suspension-grown planktonic cells.[12] Different types of strategies are used in the control and treatment of oral biofilm infections. Conventional methods of biofilm removal include mechanical removal and the use of antibacterial mouthwashes and dentifrices.[13]

Inverted amino acids have recently been found to disrupt biofilms by inducing the self-dispersal of cellular components and the matrix of the biofilm.[14] The current study investigates the impact of 5 d-amino acids (dAAs), namely d-alanine (d-ala), d-arginine (d-arg), d-leucine (d-leu), d-methionine (d-met), and d-tryptophan (d-try) on the planktonic bacterial growth, initial adhesion, biofilm formation, and biofilm dispersion of 5 early oral colonizers (*S. mitis*, *S. oralis*, *S. salivarius*, *S. gordonii*, and *S. sanguinis*) and their mixed species. There is a paucity of data on the efficacy of dAAs on biofilms formed by early colonizers.

Material and methods

D-amino acids

D-alanine, d-arg, d-leu, d-met, and d-try were purchased from Sigma-Aldrich (St. Louis, USA) (purity ≥95%). All amino acids were prepared in 100-mM working solutions by dissolving the relative weight of dAAs in distilled water or liquid culture medium, followed by filter sterilization. All dAA solutions were freshly prepared.

Bacterial cultures and media

Stock cultures of test strains, *S. gordonii* (American Type Culture Collection (ATCC) 35105), *S. mitis* (ATCC 49456), *S. oralis* (ATCC 10557), *S. sanguinis* (ATCC BAA-1455), and *S. salivarius* (ATCC 7073), were obtained from the Area of Microbiology and Immunology at the Piracicaba Dental School of State University of Campinas in Brazil. The cultures were stored in a skim milk solution (Sigma-Aldrich) at −20°C. The stock cultures were thawed and subcultured on Mitis Salivarius Agar (MSA; BD Difco™, Franklin Lakes, USA), and incubated at 37°C with 5–10% CO_2 for 24 h to obtain microbial colonies for the next steps. A few colonies of the above cultures were suspended and incubated for 24 h in sterile Brain-Heart Infusion (BHI) broth (Oxoid, Lenexa, USA), supplemented with 2% sucrose, to prepare a standard cell suspension, which was measured for absorbance at 550 nm (approx. $1×10^5$ CFU/mL).

Effect of dAAs on planktonic bacterial growth

The effect of dAAs and their concentration on planktonic bacterial cells was determined using the Clinical

and Laboratory Standards Institute (CLSI) M7-A10 broth microdilution method with AlamarBlue modification.[15] The procedure involved preparing a two-fold dilution of 100-mM working solution of dAAs (6.0–100.0 mM) in sterile BHI broth, with 100 µL/well added to 96-well sterile flat-bottom microtiter plates. Each well was inoculated with 100 µL of standard cell suspension (final Candidal cell concentration: approx. 5×10^4 CFU/mL) and incubated at 37°C and 5–10% CO_2 for 24 h.

After incubation, 10 µL of content from each well was inoculated on BHI agar plates and incubated at 37°C for 24 h to determine the minimum bactericidal concentration (MBC) of dAAs. Subsequently, 25 µL of 0.02% AlamarBlue solution was added to each well, and the plates were incubated for additional 2 h. The plates were observed visually for color changes to determine the minimum inhibitory concentration (MIC).

Effect of dAAs on bacterial adhesion under static conditions

Standard cell suspensions of test strains and their mixture were prepared in sterile, 2% sucrose-supplemented BHI broth with added 25-mM dAAs (d-ala, d-arg, d-leu, d-met, and d-try), followed by filter sterilization of the medium. Sterile polystyrene slips (diameter: 10 mm) were placed in the bottom of the 12-well cell culture cluster. The prepared cell suspension was inoculated into the 12-well cell culture cluster triplications, which were then incubated aerobically at 37°C for 6 h. After the incubation, the polystyrene slips were carefully washed with sterile distilled water to remove non-adherent cells and transferred to centrifuge tubes containing 5 mL of sterile normal saline (NS). The centrifuge tubes were then vortexed to detach adherent cells. A total of 100 µL of this inoculum was transferred to another Eppendorf tube containing 900 µL of sterile NS. Using this suspension, 10-fold dilutions (up to 10^{-8}) were prepared. Twenty-five microliters of the prepared dilutions were inoculated onto BHI agar plates in triplicate. The plates were then incubated at 37°C in a CO_2 incubator for 24 h, after which the number of colonies was counted.[16,17]

Effect of dAAs on preformed biofilms

The flow cells were assembled and sterilized using the previously described method with a few modifications.[18] The flow cells were inoculated with standard cell suspensions of test organisms and their mixture separately in the absence of 2% sucrose-supplemented sterile BHI medium. The cells were allowed to adhere to the coverslip for 6 h. After 6 h, the flow was started (0.6 rpm) and continued for another 24 h at 37°C. Then, the produced biofilms were treated with 25-mM dAA solutions for 5 min, with the flow turned off. After the treatment, the biofilms containing flow cells were washed with a flow of sterile NS, after which the biofilm density was determined by CFU assay, as previously described. The biofilm-containing coverslips were removed. Then, the biofilms were completely scraped and dissolved in 5 mL of sterile NS. The CFU assay was conducted to determine the viable biofilm biomass after dAA treatment.

Statistical analysis

The statistical analysis was carried out using the SPSS for Windows, v. 16 (SPSS Inc., Chicago, USA). A one-way analysis of variance (ANOVA) and two-way ANOVA were used to compare multiple means of more than 3 data sets. The level of significance was set at 5% ($p < 0.05$).

Results

Antimicrobial activity of dAAs

The minimum concentration of dAAs required to kill the bacterial population completely was defined as MBC. The presence of visible growth on BHI agar plates was observed after the incubation (Table 1).

There was no inhibition of planktonic bacterial growth on BHI agar after treatment with different concentrations of dAAs for 24 h.

No MIC point was noted (wells without color change of AlamarBlue stain) in any of the inverted AA samples.

Table 1. Growth of microbial cells on the surface of Brain-Heart Infusion (BHI) agar after the exposure of microbial cells to different concentrations of d-amino acids (dAAs) for 24 h

D-amino acid	S. gordonii (ATCC 35105)	S. mitis (ATCC 49456)	S. oralis (ATCC 10557)	S. sanguinis (ATCC BAA-1455)	S. salivarius (ATCC 7073)
d-alanine					
d-arginine					
d-leucine		– presence of visible growth on BHI agar plates after the incubation			
d-methionine		– no growth inhibition at any tested concentration			
d-tryptophan					

ATCC – American Type Culture Collection.

388

G. Wijesinghe et al. Inverted amino acid effect on early oral colonizers

Effect of dAAs on bacterial adhesion under static conditions

The adhered cell mass on the polystyrene surface in the presence of 25-mM dAAs was quantified using the CFU assay. Figure 1 depicts the mean colony counts of test organisms per adhered cell mass after a 6-h test period.

The adhesion of *S. mitis*, *S. oralis* and mixed species was reduced by all tested dAAs ($p < 0.05$). No adverse effects were observed on *S. gordonii* adhesion with any of the tested dAAs. Significant adhesion depletion of *S. salivarius* was observed with all tested dAAs, except for 25-mM d-arg. Only d-met and d-try were found to effectively reduce the adhesion of *S. sanguinis* onto the polystyrene surface ($p < 0.05$).

Effect of dAAs on preformed biofilms

A 24-h biofilm of test strains and their mixed species was subjected to a CFU assay, followed by treatment with 25-mM dAA solutions for 5 min. The CFU assay readings were obtained (Fig. 2). A 5-min treatment with 25-mM
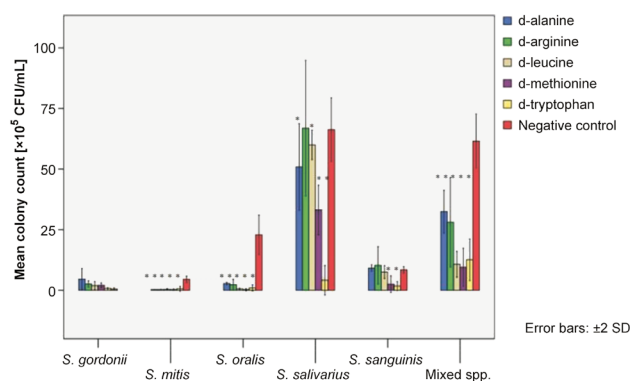
Fig. 1. Mean colony counts of *S. gordonii*, *S. mitis*, *S. oralis*, *S. salivarius*, *S. sanguinis*, and mixed species after the treatment with d-amino acids (dAAs) under static conditions

* significant decrease in adhesion compared to the negative control ($p < 0.05$). Error bars represent standard deviation (*SD*).
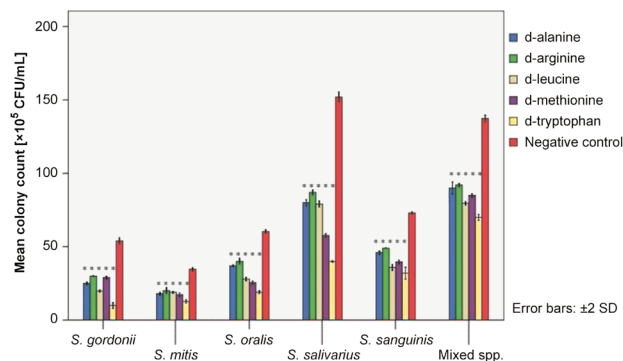
Fig. 2. Mean colony counts of *S. gordonii*, *S. mitis*, *S. oralis*, *S. salivarius*, *S. sanguinis*, and mixed species of preformed biofilms after the treatment with dAAs

* significant decrease in viable biofilm biomass compared to the negative control ($p < 0.05$). Error bars represent *SD*.

d-ala, d-arg, d-leu, d-met, and d-try resulted in a significant reduction in biofilm biomass for all test strains and their mixed species biofilms (all *p*-values <0.05).

Discussion

The oral cavity contains hundreds of different microorganisms, including bacteria, viruses and fungal species. Many of these microorganisms can associate with one another to form biofilms, which makes these organisms more resistant to mechanical stress and antibiotic agents.

The formation of oral biofilm is a dynamic process. Once the tooth surface has been cleaned, it is exposed to the fluid environment of the buccal cavity, which causes the adsorption of a thin layer of the acquired pellicle. This pellicle consists of saliva glycoproteins, mucins, statherin, agglutinin, etc.[19] The pellicle coating alters the net surface charge and free energy of oral surfaces, thereby promoting the adhesion of initial colonizers.[20] The most prevalent early colonizers of dental surfaces are Gram-positive facultative anaerobes, including *Streptococcus* spp. (*S. mitis*, *S. oralis*, *S. salivarius*, *S. gordonii*, *S. sanguinis*, etc.) and *Actinomyces* spp. These colonizers provide a basement surface for further progression of the oral biofilm. Once the early colonizers have attached to the dental surface, the biofilm develops through continued growth and subsequent adsorption of late colonizer species via coaggregation.[19] Since the transition from a healthy commensal flora towards a pathogenic flora is mediated by the progression from the initial colonizing streptococci and bacilli to the secondary colonization of late colonizers, effective control of oral biofilm formation relies on the removal of early colonizers from oral surfaces.[21] With the increased knowledge of oral biofilms, efforts to develop cost-effective, non-toxic and successful anti-biofilm strategies led to the identification of some possible biofilm control strategies. These include increasing the accessibility of antimicrobial agents to the biofilm by weakening the microbial biofilm structure, so that the action of the antimicrobial agent is more effective.[22]

Few reports point to the effects of inverted AAs on microbial biofilms. In 2010, Kolodkin-Gal et al. reported that dAAs prevent biofilm formation of *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa* by disassembling its components.[23] Another in vitro study conducted by Zilm et al. revealed the anti-biofilm effect of dAAs on *Enterococcus faecalis* biofilms in the presence of routine antimicrobials.[22]

The current study is among a select few which evaluate the effects of biofilm dispersal of inverted AAs (i.e., d-ala, d-arg, d-leu, d-met, and d-try) and their mixtures along with their antimicrobial and anti-adhesive properties using 5 early colonizing *Streptococcus* spp. (*S. mitis*, *S. oralis*, *S. salivarius*, *S. gordonii*, and *S. sanguinis*). Previous studies have shown that dAAs do not cause any

damage to the cell wall or any other cellular component. Rather, they only lead to modifications in the synthesis of peptidoglycan, a cell wall component. D-amino acids alter the chemical composition, strength and concentration of peptidoglycan by incorporating into the polymers via regulating enzymes related to peptidoglycan synthesis and modification. Since the effect of dAAs is only limited to cell wall components and it does not cause any damage to cytoplasmic compartments, the tested dAAs did not show any bactericidal or bacteriostatic effects on the test strains.[24]

However, the adhesion of *S. mitis* and *S. oralis* to the polystyrene surface was significantly reduced by all tested dAAs. The adhesion of *S. salivarius* was affected by all tested dAAs, with the exception of d-arg. The adhesion of *S. sanguinis* was not affected by d-ala, d-arg or d-leu. Furthermore, no dAA had a negative effect on the adhesion of *S. gordonii*. Interestingly, the adhesion of mixed species co-cultures was significantly diminished by all tested inverted AAs at a concentration of 25 mM. Although the adhesion of some pure cultures was not significantly reduced, this finding is of great importance, given that the oral microbial biofilms are polymicrobial in nature.

On the other hand, the cell density of mature 24-h biofilms of early colonizers was quantified after 5 min of exposure to 25-mM solution of dAAs using the CFU assay. The CFU assay demonstrated a significant reduction in the viable biofilm cell mass for treated biofilms, indicating the ability of the inverted AAs to disassemble the biomass. The mechanism of action of dAAs in inhibiting or disassembling biofilms remains unclear. Some reports suggested that dAAs interfere with cellular protein synthesis, replace d-ala in the cell wall, or act as signaling molecules that enable bacterial cells to adapt to the changing surroundings.[23,24]

Conclusions

In conclusion, the current study showed that dAAs have the potential to be used as a means of preventing the formation of oral biofilm by inhibiting the adhesion of early colonizers. Further toxicology studies and experiments are recommended to determine the effect of dAAs on mature biofilm matrix before the use of dAAs as oral rinses or oral care products.

Ethics approval and consent to participate

Not applicable.

Data availability

All data generated and/or analyzed during this study is included in this published article.

Consent for publication

Not applicable.

ORCID iDs

Gayan Kanchana Wijesinghe https://orcid.org/0000-0003-3304-9110
Malshani Nissanka https://orcid.org/0000-0002-3636-9067
Flávia Camila Maia https://orcid.org/0000-0003-1141-6661
Thaís Rossini de Oliveira https://orcid.org/0000-0001-5675-6067
José Francisco Höfling https://orcid.org/0000-0002-6054-0688

References

1. Kolenbrander PE, Andersen RN, Blehert DS, Egland PG, Foster JS, Palmer RJ Jr. Communication among oral bacteria. *Microbiol Mol Biol Rev.* 2002;66(3):486–505. doi:10.1128/MMBR.66.3.486-505.2002
2. Marsh PD, Martin MV. *Oral Microbiology.* 5th ed. Elsevier; 2009:75.
3. Vu B, Chen M, Crawford RJ, Ivanova EP. Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules.* 2009;14(7):2535–2554. doi:10.3390/molecules14072535
4. Li J, Helmerhorst EJ, Leone CW, et al. Identification of early microbial colonizers in human dental biofilm. *J Appl Microbiol.* 2004;97(6):1311–1318. doi:10.1111/j.1365-2672.2004.02420.x
5. Heller D, Helmerhorst EJ, Gower AC, Siqueira WL, Paster BJ, Oppenheim FG. Microbial diversity in the early in vivo-formed dental biofilm. *Appl Environ Microbiol.* 2016;82(6):1881–1888. doi:10.1128/AEM.03984-15
6. Rosan B, Lamont RJ. Dental plaque formation. *Microbes Infect.* 2000;2(13):1599–1607. doi:10.1016/s1286-4579(00)01316-2
7. Cook GS, Costerton JW, Lamont RJ. Biofilm formation by *Porphyromonas gingivalis* and *Streptococcus gordonii. J Periodontal Res.* 1998;33(6):323–327. doi:10.1111/j.1600-0765.1998.tb02206.x
8. Shapiro JA. Thinking about bacterial populations as multicellular organisms. *Annu Rev Microbiol.* 1998;52:81–104. doi:10.1146/annurev.micro.52.1.81
9. Marsh PD. Dental plaque: Biological significance of a biofilm and community life-style. *J Clin Periodontol.* 2005;32 Suppl 6:7–15. doi:10.1111/j.1600-051X.2005.00790.x
10. Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalized dental medicine. *Oral Dis.* 2012;18(2):109–120. doi:10.1111/j.1601-0825.2011.01851.x
11. Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. Oral microbiomes: More and more importance in oral cavity and whole body. *Protein Cell.* 2018;9(5):488–500. doi:10.1007/s13238-018-0548-1
12. Pierce CG, Uppuluri P, Tristan AR, et al. A simple and reproducible 96-well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing. *Nat Protoc.* 2008;3(9):1494–1500. doi:10.1038/nport.2008.141
13. Soukos NS, Goodson JM. Photodynamic therapy in the control of oral biofilms. *Periodontol 2000.* 2011;55(1):143–166. doi:10.1111/j.1600-0757.2010.00346.x
14. Aliashkevich A, Alvarez L, Cava F. New insights into the mechanisms and biological roles of D-amino acids in complex eco-systems. *Front Microbiol.* 2018;9:683. doi:10.3389/fmicb.2018.00683
15. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard – eleventh edition. 2018. https://clsi.org/media/1928/m07ed11_sample.pdf. Accessed December 2, 2022.
16. Bao X, de Soet JJ, Tong H, et al. *Streptococcus oligofermentans* inhibits *Streptococcus mutans* in biofilms at both neutral pH and cariogenic conditions. *PLoS One.* 2015;10(6):e0130962. doi:10.1371/journal.pone.0130962
17. Weerasekera MM, Jayarathna TA, Wijesinghe GK, et al. The effect of nutritive and non-nutritive sweeteners on the growth, adhesion, and biofilm formation of *Candida albicans* and *Candida tropicalis. Med Princ Pract.* 2017;26(6):554–560. doi:10.1159/000484718
18. Weiss Nielsen M, Sternberg C, Molin S, Regenberg B. *Pseudomonas aeruginosa* and *Saccharomyces cerevisiae* biofilm in flow cells. *J Vis Exp.* 2011;(47):2383. doi:10.3791/2383

19. Kriebel K, Hieke C, Müller-Hilke B, Nakata M, Kreikemeyer B. Oral biofilms from symbiotic to pathogenic interactions and associated disease – Connection of periodontitis and rheumatic arthritis by peptidylarginine deiminase. *Front Microbiol*. 2018;9:53. doi:10.3389/fmicb.2018.00053

20. Weerkamp AH, Uyen HM, Busscher HJ. Effect of zeta potential and surface energy on bacterial adhesion to uncoated and saliva-coated human enamel and dentin. *J Dent Res*. 1988;67(12):1483–1487. doi:10.1177/00220345880670120801

21. Ammann TW, Belibasakis GN, Thurnheer T. Impact of early colonizers on in vitro subgingival biofilm formation. *PLoS One*. 2013;8(12):e83090. doi:10.1371/journal.pone.0083090

22. Zilm PS, Butnejski V, Rossi-Fedele G, Kidd SP, Edwards S, Vasilev K. D-amino acids reduce *Enterococcus faecalis* biofilms in vitro and in the presence of antimicrobials used for root canal treatment. *PLoS One*. 2017;12(2):e0170670. doi:10.1371/journal.pone.0170670

23. Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R, Losick R. D-amino acids trigger biofilm disassembly. *Science*. 2010;328(5978):627–629. doi:10.1126/science.1188628

24. Lam H, Oh DC, Cava F, et al. D-amino acids govern stationary phase cell wall remodeling in bacteria. *Science*. 2009;325(5947):1552–1555. doi:10.1126/science.1178123

Genotypic and morphological identification of opportunistic microorganisms in triple-syringe tubing from dental units

Ninfa Ramírez-Durán[1,A,C–F], Gauddy Lizeth Manzanares-Leal[1,C–F], Josué Roberto Bermeo-Escalona[2,A–F], Rogelio José Scougall-Vilchis[2,A–F], Carlo Eduardo Medina-Solis[2,3,C–F], Pablo Antonio Moreno-Pérez[1,C–F], Blanca Silvia González-López[2,A–F]

[1] Research Laboratory of Medical and Environmental Microbiology, Autonomous University of the State of Mexico, Toluca, Mexico

[2] Center for Advanced Studies and Research in Dentistry, School of Dentistry, Autonomous University of the State of Mexico, Toluca, Mexico

[3] Academic Area of Dentistry, Institute of Health Sciences, Autonomous University of the State of Hidalgo, Pachuca, Mexico

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

Address for correspondence
Carlo Eduardo Medina-Solis
E-mail: cemedinas@yahoo.com

Cite as
Ramírez-Durán N, Manzanares-Leal GL, Bermeo-Escalona JR, et al. Genotypic and morphological identification of opportunistic microorganisms in triple-syringe tubing from dental units. *Dent Med Probl*. 2024;61(3):391–399. doi:10.17219/dmp/161187

DOI
10.17219/dmp/161187

Abstract

Background. In Mexico and around the world, water in dental units, including triple syringes, comes from municipal chlorinated water mains. The microbial contamination of dental unit water systems constitutes a risk factor for opportunistic infections.

Objectives. The present work aimed to identify the bacteria present in the triple-syringe water lines of dental units at a dental school of a public university in Mexico, with a hypothesis that opportunistic bacteria of importance to human health would be found.

Material and methods. A cross-sectional study was carried-out. A total of 100 samples of triple-syringe tubing from dental units operated by a dental school of a public university in Mexico were analyzed before and after their use in dental practice. Bacterial biofilm was cultured and isolated from the tubing, using standard microbiological methods, and then the species present were identified through *16S rRNA* gene sequencing. The characterization of the biofilm was performed by means of scanning electron microscopy (SEM).

Results. Bacterial growth was observed in 20% of the non-disinfected and 10% of the disinfected samples, with 11 strains isolated. Six genera and 11 bacterial species were genetically identified. Coagulase-negative staphylococci (CoNS), considered opportunistic human pathogens, were among the most critical microorganisms. Scanning electron microscopy revealed a thick polymeric matrix with multiple bacterial aggregates.

Conclusions. Opportunistic bacteria from human skin and mucous membranes were detected. Under normal conditions, these bacteria are incapable of causing disease, but are potentially harmful to immunosuppressed patients.

Keywords: genotype, microorganisms, opportunistic, triple syringe, dental chairs

Introduction

Biofilm formation and microbial growth in the water lines of dental units result in a great amount of microorganisms in the water used for cooling, cleaning and ultrasonic treatment in dentistry.[1] In Mexico and various places worldwide, water in dental units, including triple syringes, comes from municipal chlorinated water supply.[2] Municipal water harbors free planktonic bacteria, fungi, viruses, protozoa, unicellular algae, amebae, and nematodes, and this diversity produces multi-species biofilm. The intensity of the water flow, stagnation for more than 12 h, temperature, and the type of piping all contribute to biofilm formation and consolidation.[3]

There are few regulations to determine the acceptable level of bacteria in these environments. In Mexico, it is stated that with regard to human use and consumption, water must not contain fecal coliform bacteria or other microorganisms considered pathogenic.[4] Furthermore, it is recommended that the amount of heterotrophic bacteria in water does not exceed 200 colony-forming units (CFU) per milliliter for the water to be acceptable in dental practice.[5] Although many microorganisms are not detected in the unit inlet water, differences in the number and variety of microorganisms have been found in the outlet water, suggesting other forms of contamination, from sources such as water tanks and cisterns that supply water to dental units, bottled water used as deposits for dental units,[6] or finally the patient's or operator's skin.[7] In Mexico, water contamination has been detected in the conduction lines of dental units in studies carried out in dental schools.[8,9]

Biofilm bacteria secrete exopolysaccharides that protect them from disinfectants and antibiotics, allowing them to adhere to surfaces and acquire nutrients. The microorganisms located on the outermost part of the biofilm and fragments of the biofilm themselves can be carried by the water flow or spread by aerosols from dental work, and cause cross-contamination and infection in both healthy and immunocompromised individuals.[8,9]

Therefore, the present work aimed to identify the bacteria present in the triple-syringe water lines of dental units at a dental school of a public university in Mexico. It was hypothesized that opportunistic bacteria of importance to human health would be found in the collected samples.

Material and methods

A cross-sectional study was carried out. A total of 50 dental units from 2 postgraduate clinics of a public university in Mexico (25 from an endodontic clinic and 25 from a pediatric dentistry clinic) were examined. For this purpose, 100 triple-syringe tube segments were obtained and segregated as follows:
– disinfected samples (n = 50): The triple-syringe tubing was disinfected by passing through a 2% sodium hypochlorite solution for 2 min and letting it stay overnight. The following day, the municipal water stored in a cistern was allowed to run for 2 min, and 5-centimeter tubing samples were taken;
– non-disinfected samples (n = 50): The samples of were obtained from the same dental units, but without prior disinfection; they were taken after 3 weeks of continuous use, following standard clinic protocols.

For sampling, the external surfaces of the tubes were cleaned with 70% alcohol. The tubes were cut with a sterile scalpel, obtaining two 2.5-centimeter-long sections for bacteriological and microscopic studies. The ends of the cut sections were heat-sealed to keep the interior intact until seeding. Each sample was placed in a sterile bag, and no more than 5 h elapsed until analysis.

Bacterial isolation

The biofilm was sampled using sterile swabs. The samples were inoculated in Petri dishes with the selective culture media – Brain-Heart Infusion (BHI) Agar (BD Bioxon™, cat. No. 214700; BD, Franklin Lakes, USA), Pseudomonas Agar (cat. No. 244820; BD) and Enterococcus Agar (cat. No. 274620; BD). All plates and the 3 culture media included were incubated at 37°C for 24–48 h. Colonies that developed were isolated and purified to establish their macroscopic morphology. Gram staining was performed to determine the microscopic morphological characteristics.

Bacterial identification by *16S rRNA* gene sequence analysis

The genetic identification of the strains was performed by sequencing the *16S rRNA* gene. Biomass for this purpose was obtained by inoculating pure cultures in BHI Agar through mass streaking. The cultures were incubated for 48 h at 37°C. The biomass was then recovered by scraping the bacterial growth. Bacterial DNA was extracted using the Wizard Genomic® DNA Purification Kit (cat. No. A1120; Promega Corporation, Madison, USA) according to the manufacturer's instructions.

Once the DNA was obtained, the *16S rRNA* gene was amplified through polymerase chain reaction (PCR). The reaction mixture consisted of 3 μL of template DNA, 0.20 μL of the MyTaq polymerase mix (5 U/μL, 5 mM dNTPs (deoxynucleotide triphosphates), 15 mM MgCl$_2$) (cat. No. BIO-21105; Bioline, London, UK), 2.5 μL of the reaction buffer, and 2.5 μL (1 μM) of each primer. The following nucleotide sequences were used as universal primers: 27f: 5'-AGTTTGATCMTGCTCAG-3'; and 1492r: 5'-TACGGYTACCTTGTTACGACTT-3'.

Thermal cycling conditions for gene amplification were as follows: an initial denaturation cycle for 5 min (at 94°C); denaturation for 1 min (at 94°C); annealing for 30 s (at 59°C); and extension for 1 min (at 72°C). In total,

30 cycles were repeated, and the final extension cycle was performed for 10 min (at 72°C).

The amplified fragments were observed using 1% agarose gel (Pronadisa, cat. No. 8100.10; Condalab, Madrid, Spain) stained with ethidium bromide (cat. No. E7637-1G; Sigma-Aldrich, St. Louis, USA). Electrophoresis was performed at 120 V for 40 min. Subsequently, the amplification products were purified using the Amicon® Ultra centrifugal filtration kit (cat. No. UFC500308; Merck Millipore, Burlington, USA) and sent to Macrogen USA (Macrogen Sequencing Service; Macrogen, Rockville, USA) for sequencing.

The obtained sequences were analyzed and corrected using the ChromasPro, v. 2.6.4 (Technelysium Pty Ltd., South Brisbane, Australia), and BioEdit, v. 5.0.9,[10] programs. The consensus sequences were compared with the sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank, using the Basic Local Alignment Search Tool (BLAST) and the EzBioCloud public database.

Characterization of the biofilm

For the morphological characterization of the biofilm, 0.5-millimeter sagittal sections were cut. The samples were fixed in 0.1% glutaraldehyde, washed twice in 0.2 M cacodylate buffer at 4°C and dehydrated by increasing ethanol concentrations (from 30% to 100%). They were then dried overnight in hexamethyldisilazane (HMDS) (Electron Microscopy Sciences, Hatfield, USA), mounted in aluminum sample holders and coated with gold-palladium. A scanning electron microscope (SEM) (model 4000; Jeol, Tokyo, Japan) was used to examine the samples.

Results

Of the total of 100 triple-syringe tube segments included in this study (50 non-disinfected and 50 disinfected samples), bacterial growth occurred in 10 (20%) of the non-disinfected samples (6 samples from the endodontic clinic (12%) and 4 samples from the pediatric dentistry clinic (8%)). Moreover, growth was observed in 5 (10%) of the disinfected segments (1 dental unit from the endodontic clinic (2%) and 4 from the pediatric dentistry clinic (8%)). Bacterial growth was observed in the BHI and Pseudomonas media, although no growth was detected in the Enterococcus medium in either sample type. However, a representative of the genus *Enterococcus* was isolated in the non-selective culture medium.

Finally, the microscopic and macroscopic analysis identified 11 strains, of which 7 (64%) were from the non-disinfected samples and 4 (36%) from the disinfected samples, including 5 Gram-positive bacilli (one of them spore-forming) (45%), 3 Gram-positive cocci (27%) and 3 Gram-negative bacilli (27%), which were chosen for genetic identification by *16S rRNA* gene sequencing (Table 1).

Six genera and 11 bacterial species were detected: *Carnobacterium viridans*; *Staphylococcus epidermidis*; *Staphylococcus capitis* subsp *urealyticus*; *Bacillus gottheilii*; *Staphylococcus hominis* subsp *hominis*; *Niallia circulans*; *Bacillus safensis*; *Enterococcus quebecensis*; *Bacillus pumilus*; *Pseudomonas fluorescens*; and *Acinetobacter lwoffii* (Table 1).

A total of 64% of the identified species are opportunistic human pathogens, namely *S. epidermidis*, *S. capitis* and *S. hominis* (coagulase-negative staphylococci (CoNS)), *N. circulans*, *B. pumilus*, *P. fluorescens*, and *A. lwoffii* (Table 1).

Regarding the characterization of the biofilm by SEM, a random sample of the 5 disinfected tubes that showed bacterial growth was analyzed (40%). In some disinfected samples, there was no evidence of biofilm formation (Fig. 1A), while other disinfected samples showed biofilm formation (Fig. 1B–D).

Three random samples were analyzed from the 10 non-disinfected tubes that showed bacterial growth (30%). The non-disinfected samples showed a thick polymeric matrix layer (Fig. 2A,D,E). The detected biofilm comprised bacteria with coccoid and bacillary morphology (Fig. 2B,C), and numerous diatoms (Fig. 2F).

Discussion

The present quantitative study was conducted to evaluate contamination in triple-syringe tube sections of dental units. According to the study design, half of the tube sections were not disinfected, while the other half were previously disinfected with a 2% hypochlorite solution for 2 min. Despite prior disinfection, 5 dental units were found to be contaminated, as demonstrated by bacterial growth in the cultures and Gram staining, which was also confirmed by API (Analytical Profile Index) test galleries (data not shown). It was hypothesized that microorganisms would persist despite disinfection, since hypochlorite can reduce bacterial counts by preventing bacterial penetration into deeper layers by reacting with the surface organic matrix,[7] but it does not eliminate bacteria adhering to the surface of the tubes in the form of biofilm. A better understanding of the ecology of biofilm in water lines is needed to control biofilm formation.

According to Hoogenkamp et al., in a system such as the water lines of dental units, it is necessary that first biofilm with bacteria of the vital liquid itself is established so that the colonization of bacteria of non-aqueous origin can subsequently occur.[11] This study identified bacteria previously reported in the aquatic environment or as environmental contaminants that are not pathogenic for humans.

Table 1. Identification by *16S rRNA* gene sequencing of the bacterial species isolated from triple-syringe tubes, and the clinical importance of the bacteria

No.	Strain	Culture medium	Type of sample	Source	Molecular identification						Clinical importance	Ref.
					bp	BLAST			EzBioCloud			
						identification	cover	similarity	identification	similarity		
1	E21	BHI Agar	non-disinfected	endodontic clinic	1418	*Carnobacterium viridans* MPL-11	97%	100%	*Carnobacterium viridans* MPL-11	98.93%	non-pathogenic for humans	51
2	E31	BHI Agar	non-disinfected	endodontic clinic	1410	*Staphylococcus epidermidis* ATCC 14990(T)	99%	98%	*Staphylococcus epidermidis* ATCC 14990(T)	99.71%	human pathogen, opportunistic	19,44,52–55
3	E51	BHI Agar	non-disinfected	endodontic clinic	1403	*Staphylococcus capitis* JCM 2420	95%	99%	*Staphylococcus capitis* subsp. *urealyticus* GTC 727(T)	98.55%	human pathogen, opportunistic	21,56–59
4	E91	BHI Agar	non-disinfected	endodontic clinic	1423	*Bacillus gottheilii* WCC 4585	100%	98%	*Bacillus gottheilii* WCC 4585	100%	non-pathogenic for humans	13
5	E101	BHI Agar	non-disinfected	endodontic clinic	1412	*Staphylococcus hominis* DSM 122	98%	99%	*Staphylococcus hominis* subsp. *hominis* DSM 20328	99.57%	human pathogen, opportunistic	60–62
6	P11	BHI Agar	non-disinfected	pediatric dentistry clinic	1414	*Niallia circulans** ATCC 4513	98%	98%	*Niallia circulans** ATCC 4513	99.43%	human pathogen, opportunistic	31,35,63
7	P101	BHI Agar	non-disinfected	pediatric dentistry clinic	1409	*Bacillus safensis* NBRC 100820	98%	98%	*Bacillus safensis* FO-36b	99.22%	non-pathogenic for humans	64,65
8	E82	BHI Agar	disinfected	endodontic clinic	1435	*Enterococcus quebecensis* CCRI-16985	95%	99%	*Enterococcus quebecensis* CCRI-16985	98.53%	non-pathogenic for humans	12
9	P12A	BHI Agar	disinfected	pediatric dentistry clinic	1425	*Bacillus pumilus* ATCC 7061	99%	98%	*Bacillus pumilus* ATCC 7061	99.34%	human pathogen, opportunistic	15,16,66
10	P12B	Pseudomonas Agar	disinfected	pediatric dentistry clinic	1416	*Pseudomonas fluorescens* NBRC 14160	96%	100%	*Pseudomonas fluorescens* DSM 50090	98.45%	human pathogen, opportunistic	36–38
11	P42	BHI Agar	disinfected	pediatric dentistry clinic	1408	*Acinetobacter lwoffii* DSM 2403	97%	100%	*Acinetobacter lwoffii* NCTC 5866	98.57%	human pathogen, opportunistic	39–41

BHI – Brain-Heart Infusion; bp – base pairs; BLAST – Basic Local Alignment Search Tool; * previously called *Bacillus circulans*.
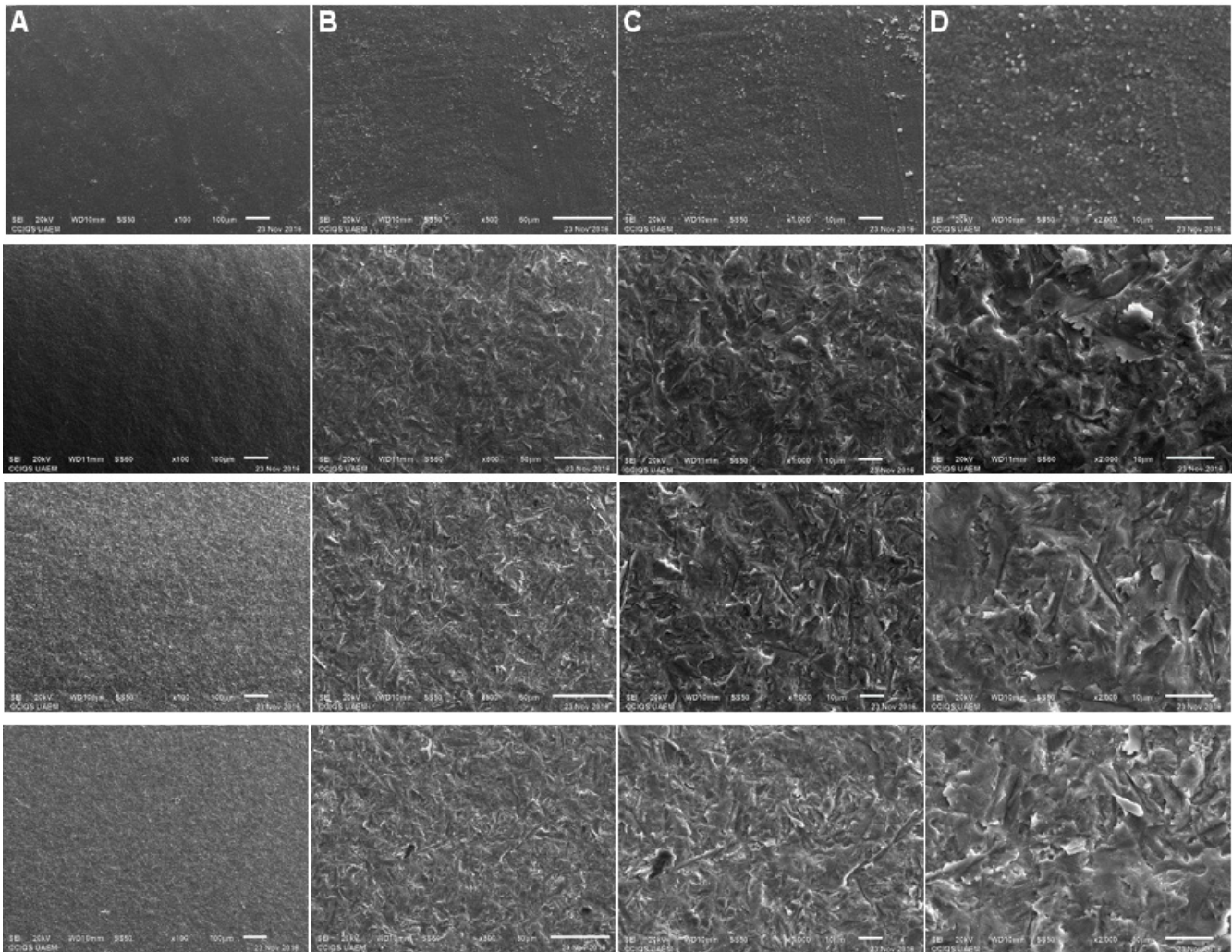
Fig. 1. Photomicrographs of disinfected triple-syringe tubes

A – no evidence of biofilm formation (×100); B–D – evidence of biofilm formation (×500, ×1,000, ×2,000, respectively).
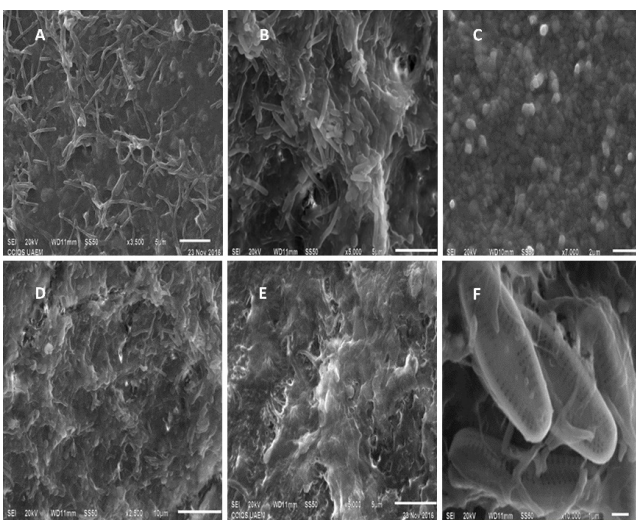
Fig. 2. Photomicrographs of non-disinfected triple-syringe tubes

A – extracellular polymeric matrix of biofilm and the bacteria separated by interstitial spaces (×3,500); B – bacilli (×5,000); C – cocci (×7,000); D – biofilm matrix and bacterial aggregates (cocci and bacilli) (×2,500); E – biofilm with a dense extracellular polymeric matrix (×5,000); F – diatoms (microscopic unicellular algae) (×10,000).

For example, *E. quebecensis*, unrelated to fecal contamination, was isolated from a water well in Canada.[12] Since it is considered a species typical of the aquatic environment free of contamination, its presence in the evaluated water lines is probably "an unusual finding". Moreover, we detected *B. gottheilii*, a species described in 2013, isolated from a pharmaceutical manufacturing site, the environmental implications of which have not yet been elucidated.[13] Therefore, we report its presence in dental unit water lines. Also, *B. safensis* was identified; it is widespread in almost the entire aquatic and terrestrial environment.[14] The bacillus *N. circulans*, an extraordinary exopolysaccharide producer,[9] probably promoted biofilm growth in the studied water lines. A related species, *B. pumilus*, was also present in the dental unit water lines, and consequently, we could consider these bacilli as "normal" environmental contaminants in the odontological ecosystem. However, this does not imply that these species are not pathogenic for humans. The specific case of *B. pumilus* has been associated with septic arthritis, food intoxication, food-borne gastroenteritis, septicemia, peritonitis, and ophthalmitis.[15,16]

As mentioned, once biofilm typical of the aquatic environment is established, it begins to colonize with bacteria of other origin. We detected bacteria from human skin and mucous membranes, such as *S. epidermidis*,[17] *S. capitis* and *S. hominis*.[18–21] All these species are CoNS, considered opportunistic bacteria composed of genes encoding adhesive factors and biofilm production factors, hemolysins, and exoenzymes.[18,19]

Coagulase-negative staphylococci, in general, can affect inpatients and outpatients, those on dialysis,[22] newborns,[23] cancer patients,[20] transplant or transfused patients,[24] those with autoimmune diseases or diseases such as acquired immune deficiency syndrome (AIDS),[25] and those with chronic diseases,[26] particularly diabetes.[21,26] The sources of contamination have been identified as skin (of the axillae, perineum and groin), membranes (of the nasal and respiratory tract)[27] and oral cavity (in both healthy and non-healthy children); CoNS are also present in patients with malignant diseases.[28] The bacteria have also been detected in human clinical specimens, purulent material, milk, and, less frequently, in blood.[29]

Niallia circulans, formerly called *Bacillus circulans*,[30] is mainly considered an opportunistic pathogen in immunocompromised patients.[31] However, many non-immunocompromised infections have been described, such as endophthalmitis,[32] mixed abscess,[31] peritonitis,[33] wound infection,[34] meningitis, and endocarditis.[35] Specific studies on the presence of this bacterium in dental settings and its correlation with non-immunocompromised infections are needed.

In the case of *P. fluorescens*, this bacterium is not generally considered a human pathogen.[36] It is widely studied for its role in the soil and the rhizosphere, but with the use of culture-independent methods, it has been found to possess several functional characteristics that enable it to inhabit mammalian hosts.[36] It has been described as causing bacteremia in humans,[37] and reports have identified it in respiratory samples.[38] It is important to monitor the presence of this bacterium, which tends to be environmental, but can easily colonize humans, with the consequences still unknown.

Within the group of bacteria detected as human pathogens, *A. lwoffii* was also found. This species is a Gram-negative aerobic bacillus that is part of the microbiota of the skin, oropharynx and perineum in about 20–25% of healthy people.[39] However, it is currently reported as an emerging pathogen associated with gastritis[40] and infections in neonatal intensive care units (ICUs).[41] Studies such as ours, in which emerging pathogens are reflected, aid in recognizing that dental units could be reservoirs of these microorganisms and, at some point, contaminate the dental work environment. Moreover, access to dental units with retrosuction valves or antibacterial materials is almost impossible in many countries, including Mexico. It is necessary to resort to low-cost resources to curb the possibility of cross-infection in the dental area.

Environmental factors, including soil, contaminated food, sewage, and animal feces, also contribute to contamination.[42] The sources of contamination in dental offices include microorganisms from the normal human skin flora of the dentist and the office staff, aerosols from patients, contamination from surfaces the operator is in contact with while caring for the patient, and biofilm from dental unit drive systems, the formation of which is favored by warm temperatures and periods of unit inactivity.[2] The water tanks of dental units supplied with distilled water have been identified as harboring various pathogenic and opportunistic microorganisms. In our study, we only observed the following, probably due to the type of sample used: *S. epidermidis*; *P. fluorescens*; and *Staphylococcus saprophyticus*.[43]

Specifically, *S. epidermidis* is a typical member of the human epithelial microbiota and one of the most frequent nosocomial pathogens. It can adhere to surfaces and form biofilm that protects it from antibiotics and host defenses; it can also detect the presence of antimicrobial peptides and respond with important defense mechanisms.[44–46] While this species does not usually cause harm to humans, it can behave as a pathogen in immunosuppressed patients. According to Heilman et al., *S. epidermidis* from the CoNS group causes chronic foreign body-related infections, increasing human morbidity and mortality, and potentially posing a problem when caring for non-immunocompetent patients in dental offices.[19]

Studies evaluating the contamination of dental unit piping systems have reported the presence of human pathogenic microorganisms.[2] The contamination degree and the identified bacterial genera depend on sociodemographic characteristics and the analyzed sample.[3,47,48]

In the present study, the microscopic characterization of the biofilm was carried out to analyze the morphology of the bacteria, the thickness of the biofilm, as well as changes in the implantation mechanisms in the previously disinfected tubes. Biofilm formation was found to be scarce, likely because little time elapsed between disinfection and measurement.

The detected biofilm showed a dense matrix with morphologically distinct bacteria, suggesting the presence of a complex structure that probably represented a remnant of mature biofilm that was not adequately removed by the chosen disinfection method.[11,49] This important finding points to a problem to be solved; it confirms that it is challenging to eradicate the biofilm formed in the aquatic environment, as it can resist antimicrobial treatment.

An unexpected finding was the presence of diatoms. They were identified through morphological analysis as *Nupela vitiosa*, described by Potapova.[50] Diatoms are recognized as one of the biological components of lotic systems most suitable for monitoring water quality in terms of organic contamination. Their presence in most of the evaluated dental units suggests the possibility of the contamination of the water used in the unit dispensers, coming from the Faculty of Dentistry cistern.[50]

Limitations

With regard to the study limitations, we can report that the number of CFU per milliliter (CFU/mL) was not quantified, which would have helped establish whether the minimum cleanliness conditions of the water entering and leaving the dental units were met. We suggest implementing this analysis in future research. Furthermore, it is necessary to evaluate representative sections along the entire length of the pipeline and the water tanks of dental units. Our inability to detect biofilm by SEM in some units while finding it in others may be due to the fixation medium, although glutaraldehyde has previously been shown to be an effective fixative. Methacarn and Carnoy's solution have also been suggested as fixatives that can preserve biofilm architecture more effectively.[50] Notably, we only considered the analysis of the biofilm collected using the swabbing method. However, we propose the use of sonication as a complementary method to recover a more significant number of bacterial species for future studies. It is important to review the data on the municipal water used in dental units and the aerosol output of dental instruments, and correlate it with oral microorganisms in patients. Finally, we propose further studies analyzing the mechanisms of bacterial resistance the reported species may have, which would help elucidate their involvement in human disease.

Conclusions

The bacteria detected in the water lines of the dental units included in this study are considered opportunistic microorganisms from the normal microbiota of the skin and mucous membranes, and the environment. Dental unit water lines are critical elements, as there are many procedures and devices in which they are used – cooling, cleaning, sterilizing, or rinsing. This water must be in the best possible condition. Studies such as this allow new questions to be raised about particular microorganisms that might be present in the dental environment. Data on this topic should be updated until total biosafety is achieved in the numerous protocols in which water is involved in dental procedures.

Ethics approval and consent to participate

The study protocol was authorized by the Institutional Review Board at the Center for Advanced Studies and Research in Dentistry, School of Dentistry, Autonomous University of the State of Mexico, Toluca, Mexico (CEICIEAO-2019-007).

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

Ninfa Ramírez-Durán https://orcid.org/0000-0003-3108-895X
Gauddy Lizeth Manzanares-Leal https://orcid.org/0000-0002-4517-2661
Josué Roberto Bermeo-Escalona https://orcid.org/0000-0003-4173-7917
Rogelio José Scougall-Vilchis https://orcid.org/0000-0003-4671-0748
Carlo Eduardo Medina-Solis https://orcid.org/0000-0002-1410-9491
Pablo Antonio Moreno-Pérez https://orcid.org/0000-0002-5755-8959
Blanca Silvia González-López https://orcid.org/0000-0002-4537-217X

References

1. Claesson R, Edwardsson S, Bäckman N. Microbial contamination of dental unit water lines – a matter of following instructions. *J Hosp Infect*. 2021;113:187–188. doi:10.1016/j.jhin.2021.04.013
2. Spagnolo AM, Sartini M, Cristina ML. Microbial contamination of dental unit waterlines and potential risk of infection: A narrative review. *Pathogens*. 2020;9(8):651. doi:10.3390/pathogens9080651
3. Alkhulaifi MM, Alotaibi DH, Alajlan H, Binshoail T. Assessment of nosocomial bacterial contamination in dental unit waterlines: Impact of flushing. *Saudi Dent J*. 2020;32(2):68–73. doi:10.1016/j.sdentj.2019.07.003
4. Diario Oficial de la Federación. NOM-201-SSA1-2002. Productos y Servicios, Agua y Hielo Para Consumo Humano, Envasados y a Granel, Especificaciones Sanitarias. 2002.
5. Diario Oficial de la Federación. NOM-013-SSA2-2015. Para la Prevención y Control de Enfermedades Bucales. 2015.
6. Mungara J, Dilna NC, Joseph E, Reddy N. Evaluation of microbial profile in dental unit waterlines and assessment of antimicrobial efficacy of two treating agents. *J Clin Pediatr Dent*. 2013;37(4):367–371. doi:10.17796/jcpd.37.4.l6851n9g01223450.
7. Wirthlin MR, Marshall GW Jr., Rowland RW. Formation and decontamination of biofilms in dental unit waterlines. *J Periodontol*. 2003;74(11):1595–1609. doi:10.1902/jop.2003.74.11.1595
8. Muñoz EJ, Hernández DD, Moreno GM. Análisis bacteriológico comparativo del agua de las clínicas urbana CLIMUZAC y rural CLITACO de la Facultad de Odontología de la Universidad Autónoma de Zacatecas. *Rev ADM*. 2006;63(1):23–31. https://www.medigraphic.com/cgi-bin/new/resumen.cgi?IDARTICULO=4844. Accessed October 6, 2021.
9. González AM, Robles VE, González VJ, Martínez PM, Loza GC, González GM. Is water in your dental unit bacteriologically safe? [In Spanish.] *Rev ADM*. 2009;66(1):16–22. https://www.medigraphic.com/cgi-bin/new/resumen.cgi?IDARTICULO=28677. Accessed October 6, 2021.
10. Hall T. BioEdit : A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser*. 1999;41:95–98. https://ci.nii.ac.jp/naid/10030689140/en. Accessed October 6, 2021.
11. Hoogenkamp MA, Brandt BW, De Soet JJ, Crielaard W. An invitro dynamic flow model for translational research into dental unit water system biofilms. *J Microbiol Methods*. 2020;171:105879. doi:10.1016/j.mimet.2020.105879
12. Sistek V, Maheux AF, Boissinot M, et al. *Enterococcus ureasiticus* sp. nov. and *Enterococcus quebecensis* sp. nov., isolated from water. *Int J Syst Evol Microbiol*. 2012;62(Pt 6):1314–1320. doi:10.1099/ijs.0.029033-0
13. Seiler H, Wenning M, Schmidt V, Scherer S. *Bacillus gottheilii* sp. nov., isolated from a pharmaceutical manufacturing site. *Int J Syst Evol Microbiol*. 2013;63(Pt 3):867–872. doi:10.1099/ijs.0.036277-0
14. Branquinho R, Sousa C, Lopes J, Pintado ME, Peixe LV, Osório H. Differentiation of *Bacillus pumilus* and *Bacillus safensis* using MALDI-TOF-MS. *PLoS One*. 2014;9(10):e110127. doi:10.1371/journal.pone.0110127
15. Shivamurthy VM, Gantt S, Reilly C, Tilley P, Guzman J, Tucker L. *Bacillus pumilus* septic arthritis in a healthy child. *Can J Infect Dis Med Microbiol*. 2016;2016:3265037. doi:10.1155/2016/3265037

16. Ozkocaman V, Ozcelik T, Ali R, et al. *Bacillus* spp. among hospitalized patients with haematological malignancies: Clinical features, epidemics and outcomes. *J Hosp Infect*. 2006;64(2):169–176. doi:10.1016/j.jhin.2006.05.014

17. Brown MM, Horswill AR. *Staphylococcus epidermidis* – Skin friend or foe? *PLoS Pathog*. 2020;16(11):e1009026. doi:10.1371/journal.ppat.1009026

18. Argemi X, Hansmann Y, Prola K, Prévost G. Coagulase-negative staphylococci pathogenomics. *Int J Mol Sci*. 2019;20(5):1215. doi:10.3390/ijms20051215

19. Heilmann C, Ziebuhr W, Becker K. Are coagulase-negative staphylococci virulent? *Clin Microbiol Infect*. 2019;25(9):1071–1080. doi:10.1016/j.cmi.2018.11.012

20. Ahmed NH, Baruah FK, Grover RK. *Staphylococcus hominis* subsp. *novobiosepticus*, an emerging multidrug-resistant bacterium, as a causative agent of septicaemia in cancer patients. *Indian J Med Res*. 2017;146(3):420–425. doi:10.4103/ijmr.IJMR_1362_15

21. Young N, Bhally H. Bilateral neck pyomyositis caused by *Staphylococcus capitis* and *Staphylococcus saccharolyticus* in a diabetic adult. *Case Rep Infect Dis*. 2017;2017:3713212. doi:10.1155/2017/3713212

22. Sahli F, Feidjel R, Laalaoui R. Hemodialysis catheter-related infection: Rates, risk factors and pathogens. *J Infect Public Health*. 2017;10(4):403–408. doi:10.1016/j.jiph.2016.06.008

23. Kimouli M, Vrioni G, Papadopoulou M, et al. Two cases of severe sepsis caused by *Bacillus pumilus* in neonatal infants. *J Med Microbiol*. 2012;61(Pt 4):596–599. doi:10.1099/jmm.0.033175-0

24. Chebbi Y, Frigui S, Raddaoui A, et al. Coagulase negative *Staphylococcus* bacteremia in hematopoietic stem cell transplant recipients: Clinical features and molecular characterization. *Acta Microbiol Immunol Hung*. 2021;68(2):73–79. doi:10.1556/030.2021.01315

25. Samje M, Yongwa O, Enekegbe AM, Njoya S. Prevalence and antibiotic susceptibility pattern of bacteriuria among HIV-seropositive patients attending the Bamenda Regional Hospital, Cameroon. *Afr Health Sci*. 2020;20(3):1045–1052. doi:10.4314/ahs.v20i3.7

26. Seget S, Rusak E, Partyka M, et al. Bacterial strains colonizing the sensor electrodes of a continuous glucose monitoring system in children with diabetes. *Acta Diabetol*. 2021;58(2):191–195. doi:10.1007/s00592-020-01601-w

27. Michalik M, Samet A, Podbielska-Kubera A, Savini V, Międzobrodzki J, Kosecka-Strojek M. Coagulase-negative staphylococci (CoNS) as a significant etiological factor of laryngological infections: A review. *Ann Clin Microbiol Antimicrob*. 2020;19(1):26. doi:10.1186/s12941-020-00367-x

28. Jackson MS. Staphylococci in the oral flora of healthy children and those receiving treatment for malignant disease. *Microb Ecol Health Dis*. 2000;12(1):60–64. doi:10.1080/089106000435617

29. Švec P, De Bel A, Sedláček I, et al. *Staphylococcus petrasii* subsp. *pragensis* subsp. nov., occurring in human clinical material. *Int J Syst Evol Microbiol*. 2015;65(7):2071–2077. doi:10.1099/ijs.0.000220

30. Gupta RS, Patel S, Saini N, Chen S. Robust demarcation of 17 distinct *Bacillus* species clades, proposed as novel *Bacillaceae* genera, by phylogenomics and comparative genomic analyses: Description of *Robertmurraya kyonggiensis* sp. nov. and proposal for an emended genus *Bacillus* limiting it only to the members of the *Subtilis* and *Cereus* clades of species. *Int J Syst Evol Microbiol*. 2020;70(11):5753–5798. doi:10.1099/ijsem.0.004475

31. Russo A, Tarantino U, D'Ettorre G, et al. First report of spondylodiscitis caused by *Bacillus circulans* in an immunocompetent patient: Clinical case and review of the literature. *IDCases*. 2021;23:e01058. doi:10.1016/j.idcr.2021.e01058

32. Tandon A, Tay-Kearney ML, Metcalf C, McAllister L. *Bacillus circulans* endophthalmitis. *Clin Experiment Ophthalmol*. 2001;29(2):92–93. PMID:11341454.

33. Berry N, Hassan I, Majumdar S, Vardhan A, McEwen A, Gokal R. *Bacillus circulans* peritonitis in a patient treated with CAPD. *Perit Dial Int*. 2004;24(5):488–489. PMID:15490992.

34. Logan NA, Old DC, Dick HM. Isolation of *Bacillus circulans* from a wound infection. *J Clin Pathol*. 1985;38(7):838–839. doi:10.1136/jcp.38.7.838

35. Sanyal SK, Karmaker M, Sultana M, Hossain MA. Association of *Bacillus circulans* with non-diabetic foot infection in Bangladeshi patient. *Indian J Med Microbiol*. 2015;33(4):606–608. doi:10.4103/0255-0857.167346

36. Scales BS, Dickson RP, LiPuma JJ, Huffnagle GB. Microbiology, genomics, and clinical significance of the *Pseudomonas fluorescens* species complex, an unappreciated colonizer of humans. *Clin Microbiol Rev*. 2014;27(4):927–948. doi:10.1128/CMR.00044-14

37. Benito N, Mirelis B, Luz Gálvez M, et al. Outbreak of *Pseudomonas fluorescens* bloodstream infection in a coronary care unit. *J Hosp Infect*. 2012;82(4):286–289. doi:10.1016/j.jhin.2012.09.008

38. Liu X, Xiang L, Yin Y, Li H, Ma D, Qu Y. Pneumonia caused by *Pseudomonas fluorescens*: A case report. *BMC Pulm Med*. 2021;21(1):212. doi:10.1186/s12890-021-01573-9

39. Rathinavelu S, Zavros Y, Merchant JL. *Acinetobacter lwoffii* infection and gastritis. *Microbes Infect*. 2003;5(7):651–657. doi:10.1016/s1286-4579(03)00099-6

40. Sung JJY, Coker OO, Chu E, et al. Gastric microbes associated with gastric inflammation, atrophy and intestinal metaplasia 1 year after *Helicobacter pylori* eradication. *Gut*. 2020;69(9):1572–1580. doi:10.1136/gutjnl-2019-319826

41. Mittal S, Sharma M, Yadav A, Bala K, Chaudhary U. *Acinetobacter lwoffii* an emerging pathogen in neonatal ICU. *Infect Disord Drug Targets*. 2015;15(3):184–188. doi:10.2174/1871526515666150826114745

42. Becker K, Heilmann C, Peters G. Coagulase-negative staphylococci. *Clin Microbiol Rev*. 2014;27(4):870–926. doi:10.1128/CMR.00109-13

43. Szymańska J, Sitkowska J. Opportunistic bacteria in dental unit waterlines: Assessment and characteristics. *Future Microbiol*. 2013;8(5):681–689. doi:10.2217/fmb.13.33

44. Otto M. *Staphylococcus epidermidis* – the 'accidental' pathogen. *Nat Rev Microbiol*. 2009;7(8):555–567. doi:10.1038/nrmicro2182

45. Banaszkiewicz S, Calland JK, Mourkas E, Sheppard SK, Pascoe B, Bania J. Genetic diversity of composite enterotoxigenic *Staphylococcus epidermidis* pathogenicity islands. *Genome Biol Evol*. 2019;11(12):3498–3509. doi:10.1093/gbe/evz259

46. Tröger B, Heidemann M, Osthues I, et al. Modulation of *S. epidermidis*-induced innate immune responses in neonatal whole blood. *J Microbiol Immunol Infect*. 2020;53(2):240–249. doi:10.1016/j.jmii.2018.04.008

47. Fotedar S, Ganju S. Microbial contamination of dental unit water lines in H.P. Government Dental College, Shimla. *Saudi J Dent Res*. 2015;6(2):129–132. doi:10.1016/j.sjdr.2014.11.002

48. Ehrhardt J, Alabi AS, Kremsner PG, et al. Bacterial contamination of water samples in Gabon, 2013. *J Microbiol Immunol Infect*. 2017;50(5):718-722. doi:10.1016/j.jmii.2016.03.009

49. Singh R, Stine OC, Smith DL, Spitznagel JK Jr. Labib ME, Williams HN. Microbial diversity of biofilms in dental unit water systems. *Appl Environ Microbiol*. 2003;69(6):3412–3420. doi:10.1128/AEM.69.6.3412-3420.2003

50. Potapova M. *Nupela vitiosa*. In: Diatoms of North America. 2010. https://diatoms.org/species/nupela_vitiosa. Accessed October 6, 2021.

51. Dassanayake RP, Falkenberg SM, Stasko JA, Shircliff AL, Lippolis JD, Briggs RE. Identification of a reliable fixative solution to preserve the complex architecture of bacterial biofilms for scanning electron microscopy evaluation. *PLoS One*. 2020;15(5):e0233973. doi:10.1371/journal.pone.0233973

52. Holley RA, Guan TY, Peirson M, Yost CK. *Carnobacterium viridans* sp. nov., an alkaliphilic, facultative anaerobe isolated from refrigerated, vacuum-packed bologna sausage. *Int J Syst Evol Microbiol*. 2002;52(Pt 5):1881–1885. doi:10.1099/00207713-52-5-1881

53. Kleinschmidt S, Huygens F, Faoagali J, Rathnayake IU, Hafner LM. *Staphylococcus epidermidis* as a cause of bacteremia. *Future Microbiol*. 2015;10(11):1859–1879. doi:10.2217/fmb.15.98

54. Otto M. *Staphylococcus epidermidis* pathogenesis. *Methods Mol Biol*. 2014;1106:17–31. doi:10.1007/978-1-62703-736-5_2

55. Vuong C, Otto M. *Staphylococcus epidermidis* infections. *Microbes Infect*. 2002;4(4):481–489. doi:10.1016/s1286-4579(02)01563-0

56. Oliveira F, Rohde H, Vilanova M, Cerca N. Fighting *Staphylococcus epidermidis* biofilm-associated infections: Can iron be the key to success? *Front Cell Infect Microbiol*. 2021;11:798563. doi:10.3389/fcimb.2021.798563

57. Thakker RA, Chatila K, Reynoso D, Karnath B. Native and prosthetic valve *Staphylococcus capitis* endocarditis: A review of the literature. *Cardiol Res*. 2021;12(3):140–145. doi:10.14740/cr1231

58. Tchana-Sato V, Defraigne JO. *Staphylococcus capitis* causing infective endocarditis: Not so uncommon. *Infection*. 2020;48(6):975–977. doi:10.1007/s15010-020-01514-4

59. Al Hennawi HET, Mahdi EM, Memish ZA. Native valve *Staphylococcus capitis* infective endocarditis: A mini review. *Infection.* 2020;48(1):3–5. doi:10.1007/s15010-019-01311-8

60. Tevell S, Hellmark B, Nilsdotter-Augustinsson Å, Söderquist B. *Staphylococcus capitis* isolated from prosthetic joint infections. *Eur J Clin Microbiol Infect Dis.* 2017;36(1):115–122. doi:10.1007/s10096-016-2777-7

61. Reydams H, Wuytack A, Piepers S, et al. Genetic diversity and iron metabolism of *Staphylococcus hominis* isolates originating from bovine quarter milk, rectal feces, and teat apices. *J Dairy Sci.* 2022;105(12):9995–10006. doi:10.3168/jds.2022-22216

62. Vasconcellos D, Weng B, Wu P, Thompson G, Sutjita M. *Staphylococcus hominis* infective endocarditis presenting with embolic splenic and renal infarcts and spinal discitis. *Case Rep Infect Dis.* 2022;2022:7183049. doi:10.1155/2022/7183049

63. Azimi T, Mirzadeh M, Sabour S, Nasser A, Fallah F, Pourmand MR. Coagulase-negative staphylococci (CoNS) meningitis: A narrative review of the literature from 2000 to 2020. *New microbes New Infect.* 2020;37:100755. doi:10.1016/j.nmni.2020.100755

64. Alebouyeh M, Gooran Orimi P, Azimi-Rad M, et al. Fatal sepsis by *Bacillus circulans* in an immunocompromised patient. *Iran J Microbiol.* 2011;3(3):156–158. PMID:22347600. PMCID:PMC3279812.

65. Prakash J, Arora NK. Novel metabolites from *Bacillus safensis* and their antifungal property against *Alternaria alternata*. *Antonie Van Leeuwenhoek.* 2021;114(8):1245–1258. doi:10.1007/s10482-021-01598-4

66. Lateef A, Adelere IA, Gueguim-Kana EB. The biology and potential biotechnological applications of *Bacillus safensis*. *Biologia.* 2015;70(4):411–419. doi:10.1515/biolog-2015-0062

Correlation between bilateral TMJ MRI findings: A systematic review of the literature

Nicolò Giuseppe Sorrenti[A–C], Daniele Manfredini[E,F], Filippo Sornig[B], Marco Ferrari[F], Anna Colonna[C,E], Matteo Val[A,C–E]

Department of Medical Biotechnologies, School of Dental Medicine, University of Siena, 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

Address for correspondence
Matteo Val
E-mail: matteo.val@outlook.it

Abstract

The correlation between magnetic resonance imaging (MRI) signs and clinical findings has been high-lighted in multiple studies. However, very little information is available on the correlation between the bilateral temporomandibular joints (TMJs) of the same individual. The majority of efforts in the clinical research setting have focused on the correlation between ipsilateral imaging and clinical findings, while less attention has been paid to the contralateral imaging findings of the anatomical structures.

The objective of this paper was to review the existing literature that compares temporomandibular joint (TMJ) magnetic resonance imaging (MRI) findings from both sides of the same individual.

In January 2024, a systematic search of the literature from major search engines (MEDLINE (PubMed), Scopus) was conducted to identify all peer-reviewed English-language studies that presented an MRI comparison of left and right TMJ data in the same patients. The articles were analyzed using a Population/Intervention/Comparison/Outcome (PICO) format.

The search terms "temporomandibular joint" AND "magnetic" AND "resonance" yielded 2,561 results. Only 2 papers met the established inclusion criteria. The results of the papers included in the systematic review were not comparable due to differences in the evaluation of the TMJs, which prevented a meta-analysis. Manfredini et al. identified a statistical correlation between disc displacement, osseous changes (OC) and joint effusion (JE) between the joints of the contralateral sides. Koca et al. observed a significant difference in TMJ MRI findings between the painful and non-painful sides of each individual in a bruxism group and a control group ($p = 0.001$, $p < 0.001$ and $p = 0.004$, $p < 0.001$, respectively).

The studies on the correlation between the right and left TMJs remain scarce. A comparative analysis of the 2 sides of the TMJ in individual patients is rarely reported. The review did not identify a common result for the findings of the contralateral TMJs in the 2 articles included.

Keywords: magnetic resonance, temporomandibular joint disorder, bilateral evaluation

Introduction

The temporomandibular joint (TMJ) is a fundamental structure of the stomatognathic system and is composed of several components, including the articular fossa, articular eminence, disc, and mandibular condyle. When there is an issue with the TMJ and/or the masticatory muscles, the resulting musculoskeletal symptoms are known as temporomandibular disorders (TMD).[1,2] Temporomandibular disorders are the most common non-odontogenic cause of orofacial pain and include cardinal symptoms such as pain, limited jaw movement and joint noises, which may have a negative impact on an individual's quality of life.[3]

The use of medical imaging is important for the differential diagnosis of TMD. Consequently, studies on the correlation between imaging and clinical findings are of paramount importance in order to gain a better insight into an effective prescription. Although conventional radiography can provide images of the TMJ bony components, it offers a bidimensional evaluation that is often obstructed by other parts of the cranium. Currently, cone beam computed tomography (CBCT) and magnetic resonance imaging (MRI) have emerged as the reference imaging techniques.[4–6] In particular, MRI is the gold standard for visualizing soft tissues of the TMJ and the presence of joint effusion (JE).[7] With recent advancements in MRI hardware and techniques, dynamic imaging is now possible for MRI of TMD with significantly improved image quality.[8,9]

The correlation of MRI signs with clinical findings has been highlighted in multiple studies.[10–14] However, very little information is available on the correlation between the bilateral TMJs of the same individual.[13] The majority of efforts in the clinical research setting have been directed towards investigating the correlation between ipsilateral imaging and clinical findings, while less focus has been put on the contralateral imaging findings of the anatomical structures. The ability to identify imaging signs associated with specific findings in the joints on the opposite sides of the body is potentially important from a clinical-pathological perspective, as it contributes to understanding the clinical relevance of specific imaging signs with respect to the presence of symptoms.[15,16] This is especially interesting when considering that the TMJs are a unique example of connected units.[17] Based on these premises, the intent of this manuscript was to systematically review all of the publications correlating right and left TMJs in the same individual. The primary objective of the review was to assess the relationship between TMJ disorders diagnosed using MRI of the joints of both sides of the same individual.

Material and methods

Search strategy

A systematic search of the literature was conducted to identify all peer-reviewed English-language articles that were crucial for the topic of the review: the investigation of right and left TMJs in an individual patient by means of MRI. As a starting point, we conducted a search using Medical Subjects Headings (MeSH) terms in the National Library of Medicine, MEDLINE (PubMed) database. The following terms were adopted and inserted in the search bar: "temporomandibular joint" AND "magnetic" AND "resonance" ("temporomandibular joint" [MeSH terms] OR ("temporomandibular" [all fields] AND "joint" [all fields]) OR "temporomandibular joint" [all fields]) AND ("magnetic resonance spectroscopy" [MeSH terms] OR ("magnetic" [all fields] AND "resonance" [all fields] AND "spectroscopy" [all fields]) OR "magnetic resonance spectroscopy" [all fields] OR ("magnetic" [all fields] AND "resonance" [all fields]) OR "magnetic resonance" [all fields]). The results were sorted using the Best Match research engine filter. Next, an article screening strategy was implemented by excluding all the articles whose titles were not relevant to the search query. Subsequently, additional publications were excluded based on their abstracts, while the remaining articles were ultimately excluded following a full-text review. Finally, the search was expanded through the use of the "PubMed related articles" section and a manual search of the Scopus database.

Inclusion criteria

The following inclusion criteria were applied: articles written in English; articles presenting the investigation of TMJs through the use of MRI; articles correlating findings or specifically reporting findings from the 2 contralateral sides at the individual level.

Exclusion criteria

The following publications were excluded from the analysis: systematic reviews or meta-analyses; non-systematic reviews; case reports; studies reporting data from previous publications; opinion papers; letters to the editor; and articles published before the year 2000.

Selection of participants

The participants of the included studies were adults of both genders diagnosed with any type of TMJ disorder. The individuals underwent an MRI examination of both TMJs.

Structured reading

A Population/Intervention/Comparison/Outcome (PICO) strategy was employed to read and investigate the selected articles. The study population ("P") was described in every article according to the inclusion criteria, the demographic features of the sample and the sample size. The study intervention data ("I") was collected based on all procedural characteristics of the study, including the type of intervention/experiment, assessment instruments and statistical analysis. The presence of a comparison group ("C"), such as a control group or another comparison subset among the patient population, was documented within the comparison criterion. The study outcome ("O") was evaluated in light of the need to assess a potential relationship between the TMJs of the left and right sides within the same patient. Given the extensive temporal range of the articles selected for the review, it was not feasible to structure the review according to the PICOT framework, where "T" represents the time frame.

Two of the authors participated in the stepwise search process (NGS and FS), independently reviewing titles and abstracts of all articles and then conducting a full-text screening. Two authors (MV and AC) supervised the activity and intervened in cases of uncertainty regarding the potential inclusion of an article or data interpretation. Once the review team had reached a consensus on the articles to be included in the review, the main reviewer (NGS) proceeded to perform data extraction based on the abovementioned PICO strategy. The initial plan was to conduct a meta-analysis of the data in the event that sufficient and homogeneous material was retrieved.

Estimation of the quality of studies

Grading of the level of evidence was based on the work of David Sackett and his team, as described by Richardson.[18] The classification made by Sackett et al.[19] is presented in Table 1.

Results

The literature search yielded 2,561 results, of which 2,020 were excluded due to duplication. Of the 541 remaining articles, only 7 were deemed potentially suitable for inclusion based on the assessment of abstracts. All 7 articles were read in full, and two[13,20] matched the eligibility criteria and were included in the study (Fig. 1).

The main characteristics of the included articles are reported in Table 2.

Diagnostic equipment and MRI type

In both studies,[13,20] 1.5 Tesla MRI scanners from 2 different manufacturers (GE Medical Systems, Buc, France;

Table 1. Levels of evidence based on the study by Sackett et al.[19]

Level of evidence	Study design
I	• systematic reviews of RCTs • large RCTs (with narrow confidence intervals) ($N > 100$)
II	• systematic reviews of cohort studies • outcomes research (very large ecologic studies) • small RCTs (with wide confidence intervals) ($N < 100$)
III	• cohort studies with a concurrent control group • systematic reviews of case-control studies
IV	• cohort studies without a concurrent control group (e.g., with a historical control group) • case series • case-control studies
V	• expert opinion case studies or report bench research • expert opinion based on the theory of physiologic research • common sense/anecdotes

RCT – randomized controlled trial.
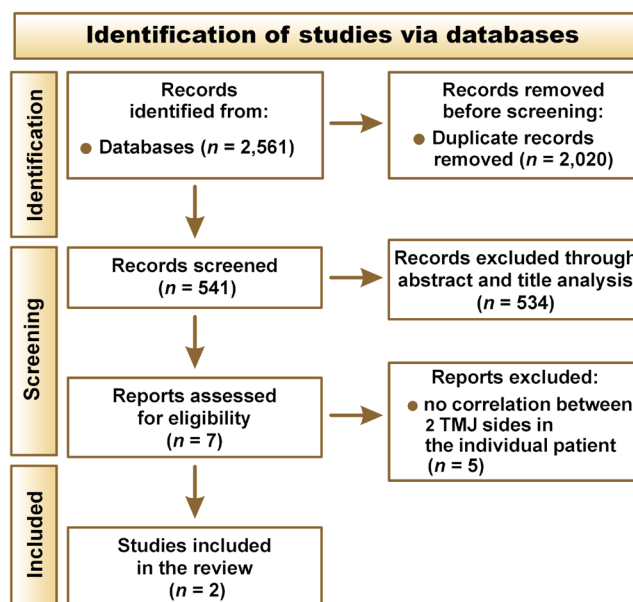
Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart of article selection process

Siemens Healthcare GmbH, Erlangen, Germany) were involved in the diagnostic process. Both publications employed T1- and T2-weighted sequences for the evaluation of patients. In both studies,[13,20] participants underwent MRI analysis with open and closed mouth positions.

Study population

A total of 478 patients (300 female) were included in the 2 studies. The patients exhibited a range of TMD, and in both investigations, the following findings were evaluated: disc position; disc morphology; and osseous changes (OC).[13,20] Additionally, both Manfredini et al.[13] and Koca et al.[20] investigated the presence of effusion and fluid accumulation.

Table 2. Summary of the selected studies based on the Population/Intervention/Comparison/Outcome (PICO) framework

Variable		Manfredini et al.[13]	Koca et al.[20]
Year of publication		2014	2024
Country		Italy	Turkey
Population	total number of patients	199	279
	clinical diagnosis	TMJ pain and dysfunction	TMJ pain and dysfunction
Intervention	diagnostic equipment	1.5 Tesla (GE Signa Contour; GE Medical Systems, Buc, France)	1.5 Tesla Symphony or Avanto MRI instruments (Siemens Healthcare GmbH, Erlangen, Germany)
	statistical analysis	multivariate permutation test with Tippett's combination method, single-variable correlation matrix, χ^2 test	Kolmogorov–Smirnov test, Mann–Whitney U test, χ^2 test, Cohen's kappa test
	MRI type	sequential gradient echo T1-weighted and fast STIR T2-weighted bilateral images for open and closed mouth	T1- and T2-weighted sequences for open and closed mouth
Comparison of the 2 TMJs		superior (normal) disc position, DDR, DDNR, presence or absence of osteoarthritis, presence and type of effusion (inflammatory fluid accumulation)	anterior DDR, anterior DDNR, posterior disc displacement, normal or abnormal articular disc morphology, condylar degeneration, presence and type of effusion (inflammatory fluid accumulation)
Outcome		• correlation between the presence of DDNR and osseous changes at the same side • the same pattern of MRI signs is expected to characterize 2 sides of the TMJ	• significant differences in disc position, disc form, condyle shape, and effusion between the sides with and without pain for both control ($p < 0.001$) and bruxism groups ($p = 0.001$, $p < 0.001$, $p = 0.004$, and $p < 0.001$, respectively)

MRI – magnetic resonance imaging; TMJ – temporomandibular joint; STIR – short tau inversion recovery; DDR – disc displacement with reduction; DDNR – disc displacement without reduction.

Quality of studies

Table 3 presents the level of evidence for the 2 selected studies. The level of evidence was considered medium-low (III), given that both papers were cohort studies with a control group.[13,20]

Comparison of right and left TMJs

Koca et al. compared TMJ MRI findings between painful and non-painful sides in TMD patients, distinguishing between bruxers and non-bruxers.[20] Significant differences in the disc/condyle structure and effusion were present between the painful and non-painful sides of each patient ($p < 0.05$). The study demonstrated an increased prevalence of anterior disc with reduction, anterior disc without reduction, and effusion in patients exhibiting an abnormal disc/condyle relationship on the painful side, regardless of the group they belonged to (bruxism or no bruxism group). Manfredini et al. compared MRI signs on the left and right sides of the TMJ.[13] The study found that if a specific diagnosis was present on one side, it was likely to be present on the opposite side as well. However, the study did not report on patient symptoms. The correlation level was moderate for disc displacement with reduction (DDR) (R = 0.47) and OC (R = 0.50). Additionally, the study found negative correlations between OC and JE, as well as between DDR and disc displacement without reduction (DDNR) in the opposite joint. The results of the global multivariate permutation test with Tippett's combination method indicated that the null hypothesis of independence between the diagnoses of the 2 sides was rejected in favor of the hypothesis of positive global

Table 3. Level of evidence of the selected studies

Study	Level of evidence
Manfredini et al.[13]	III
Koca et al.[20]	

association at the α significance level of 0.001. The adjusted *p*-values exceeded the significance level, with the exception of the tests where a specific symptom on one side was compared with the same symptom on the other side.

Statistical analysis

Due to the limited number of available studies, it was not feasible to perform a meta-analysis for the purposes of this systematic review. Instead, a descriptive analysis of the studies was conducted.

Discussion

The distinctive characteristics of the TMJs have posed significant challenges to their study, necessitating the development of tailored approaches. In fact, the coordinated action of the right and left joints ensures the forward movement of the entire anatomical structure. Given that the 2 TMJs are non-disjointed structures, an interesting issue arises regarding the potential for pathologies in one joint to also affect the contralateral joint.[2,13,21,22] To date, there has been a paucity of studies addressing the concurrent contralateral findings.[23,24] Indeed, the majority of literature on TMD describes individual cases and focuses on the correlation between clinical and imaging results rather than on the bilateral joint findings.[25]

This paper presents a review of the articles that best address the topic of contralateral findings. In analyzed studies,[13,20] the participants were selected based on their TMD symptoms, even if some differences in the recruitment strategies prevented the findings from being combined. Indeed, Koca et al. included patients with unilateral preauricular pain and divided them into those with and without bruxism.[20] Manfredini et al. did not provide any information on the concurrent presence of pain symptoms, which was beyond the scope of that paper.[13] Both investigations employed similar 1.5 Tesla MRI machines for the evaluation of the TMJs. Images were acquired with the patient in the position of maximum mouth opening and with the mouth closed.

A previous study conducted by Takahara et al. revealed significant associations between pain, disc displacement, effusion, and degenerative changes.[26] In the research carried out by Koca et al., the frequencies of intraarticular degenerative changes, effusion, DDR, and DDNR were significantly higher on the painful side of each patient compared to the non-painful side in both the control and bruxism groups.[20] In accordance with previous studies,[27,28] the findings of Koca et al.[20] revealed that the frequencies of abnormal disc/condyle relationships were significantly higher on the painful side of each patient in both the control and bruxism groups in comparison to normal disc/condyle relationships ($p < 0.05$). Additionally, significant differences were observed in the disc/condyle structure and effusion between the painful and non-painful sides of each patient in both groups ($p < 0.05$).[20] In a study by Manfredini et al., it was found that diagnoses on one side of the TMJ were usually associated with the same diagnoses on the other side, pointing to a mutual interaction between the joints on opposite sides of the body.[13] This is consistent with the theory that an imbalance between the loads exerted on the joint (such as prolonged jaw clenching) and the joint resistance (structural anatomy) can affect the TMJs bilaterally.[17] This finding is a novel addition to the literature on the need to report the results of clinical assessments of the bilateral joints.[23,24] A study conducted by Görürgöz et al. on the TMJs of 258 patients using CBCT revealed that 209 out of 258 cases had bilateral condylar bone changes.[29] The same study found no statistically significant difference between the types of degenerative changes on the right and left TMJs, as determined by McNemar's test ($p = 0.668$). This supports the findings of Manfredini et al., which demonstrated an association between the diagnoses assigned to both TMJ sides.[13]

In addition, both studies[13,20] identified a correlation between DDNR and degenerative disease within the same joints. This supports the idea that the TMJ disc plays a protective role in preventing remodeling or damage to the articular bone structures. According to several papers,[30–32] long-lasting displacement of the disc is associated with degenerative changes in the bone. As both studies are observational in nature, it is not possible to determine the

causative factor responsible for joint degeneration originating from DDNR. However, the presence of DDNR may increase the risk of osteoarthrosis in the TMJs.[33]

This review focused on radiological signs observed on MRI scans of both sides of the TMJ. However, the conflicting results between the 2 included studies make it difficult to reach consistent conclusions. This may be due to the different criteria adopted for patient recruitment. The discrepancies in the selection of the study group, especially in the study by Koca et al., where 40.1% of the patients did not have bruxism or other systemic pathologies that could have led to joint degeneration, may have contributed to the partial inconsistency of the findings.[20] On the other hand, Manfredini et al. did not provide any details regarding bruxism behaviors in the study group.[13] Therefore, further investigation is needed to determine whether and how prolonged bruxism activities may influence the contralateral findings. From a clinical perspective, it is important to consider the presence of symptoms on one or both sides when interpreting the results of these studies. The prevalence of unilateral and bilateral clinical TMD symptoms and their frequency in patient populations have not yet been determined.[23] It is plausible to suggest that certain patients may exhibit identical MRI findings on both sides despite experiencing symptoms on only one side, or the opposite may be true. This hypothesis is consistent with prior research indicating that the level of agreement between clinical and imaging diagnoses is not always flawless. Future research is required to ascertain the significance of imaging signs in the absence of clinical symptoms.

Conclusions

The limited number of papers and partially conflicting results precluded any definitive observation about the correlation between the 2 sides of the TMJ. It is a common practice in the international literature to consider only 1 TMJ for clinical research studies, as evidenced by the majority of publications. In light of these considerations, further research on the topic of contralateral findings is necessary in order to expand knowledge on the clinical relevance of MRI data.

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.

406

N.G. Sorrenti et al. Correlation between bilateral TMJ MRI findings

ORCID iDs

Nicolò Giuseppe Sorrenti ☉ https://orcid.org/0009-0002-1191-5444
Daniele Manfredini ☉ https://orcid.org/0000-0001-6642-6002
Filippo Sornig ☉ https://orcid.org/0009-0008-3431-4389
Marco Ferrari ☉ https://orcid.org/0000-0001-5375-076X
Anna Colonna ☉ https://orcid.org/0000-0002-5869-2068
Matteo Val ☉ https://orcid.org/0000-0002-4574-4391

References

1. Scrivani SJ, Keith DA, Kaban LB. Temporomandibular disorders. *N Engl J Med*. 2008;359(25):2693–2705. doi:10.1056/NEJMra0802472
2. Ahmad M, Hollender L, Anderson Q, et al. Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD): Development of image analysis criteria and examiner reliability for image analysis. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;107(6):844–860. doi:10.1016/j.tripleo.2009.02.023
3. Dahlström L, Carlsson GE. Temporomandibular disorders and oral health-related quality of life. A systematic review. *Acta Odontol Scand*. 2010;68(2):80–85. doi:10.3109/00016350903431118
4. Almeida FT, Pacheco-Pereira C, Flores-Mir C, Le LH, Jaremko JL, Major PW. Diagnostic ultrasound assessment of temporomandibular joints: A systematic review and meta-analysis. *Dentomaxillofac Radiol*. 2019;48(2):20180144. doi:10.1259/dmfr.20180144
5. Larheim TA, Abrahamsson AK, Kristensen M, Arvidsson LZ. Temporomandibular joint diagnostics using CBCT. *Dentomaxillofac Radiol*. 2015;44(1):20140235. doi:10.1259/dmfr.20140235
6. Matsubara R, Yanagi Y, Oki K, et al. Assessment of MRI findings and clinical symptoms in patients with temporomandibular joint disorders. *Dentomaxillofac Radiol*. 2018;47(4):20170412. doi:10.1259/dmfr.20170412
7. Tomas X, Pomes J, Berenguer J, et al. MR imaging of temporomandibular joint dysfunction: A pictorial review. *Radiographics*. 2006;26(3):765–781. doi:10.1148/rg.263055091
8. Karlo CA, Patcas R, Kau T, et al. MRI of the temporo-mandibular joint: Which sequence is best suited to assess the cortical bone of the mandibular condyle? A cadaveric study using micro-CT as the standard of reference. *Eur Radiol*. 2012;22(7):1579–1585. doi:10.1007/s00330-012-2391-8
9. Yoshida M, Honda E, Ozawa E, et al. Principles of the magnetic resonance imaging movie method for articulatory movement. *Oral Radiol*. 2019;35(2):91–100. doi:10.1007/s11282-018-0347-7
10. Katzberg RW, Westesson PL, Tallents RH, Drake CM. Anatomic disorders of the temporomandibular joint disc in asymptomatic subjects. *J Oral Maxillofac Surg*. 1996;54(2):147–153; discussion 153–155. doi:10.1016/s0278-2391(96)90435-8
11. Larheim TA. Role of magnetic resonance imaging in the clinical diagnosis of the temporomandibular joint. *Cells Tissues Organs*. 2005;180(1):6–21. doi:10.1159/000086194
12. Manfredini D, Basso D, Salmaso L, Guarda-Nardini L. Temporomandibular joint click sound and magnetic resonance-depicted disk position: Which relationship? *J Dent*. 2008;36(4):256–260. doi:10.1016/j.jdent.2008.01.002
13. Manfredini D, Bonnini S, Stellini E, Salmaso L, Guarda-Nardini L. Comparison of magnetic resonance imaging findings in temporomandibular joints of the two sides. *Clin Oral Investig*. 2014;18(2):499–506. doi:10.1007/s00784-013-0984-5
14. Manfredini D, Guarda-Nardini L. Agreement between Research Diagnostic Criteria for Temporomandibular Disorders and magnetic resonance diagnoses of temporomandibular disc displacement in a patient population. *Int J Oral Maxillofac Surg*. 2008;37(7):612–616. doi:10.1016/j.ijom.2008.04.003
15. Güler N, Yatmaz PI, Ataoglu H, Emlik D, Uckan S. Temporomandibular internal derangement: Correlation of MRI findings with clinical symptoms of pain and joint sounds in patients with bruxing behaviour. *Dentomaxillofac Radiol*. 2003;32(5):304–310. doi:10.1259/dmfr/24534480
16. Roh HS, Kim W, Kim YK, Lee JY. Relationships between disk displacement, joint effusion, and degenerative changes of the TMJ in TMD patients based on MRI findings. *J Craniomaxillofac Surg*. 2012;40(3):283–286. doi:10.1016/j.jcms.2011.04.006
17. Manfredini D, ed. *Current Concepts on Temporomandibular Disorders*. 1st ed. New Malden, UK: Quintessence; 2010:207–222.
18. Richardson PEH. David Sackett and the birth of evidence based medicine: How to practice and teach EBM. *BMJ*. 2015;350:h3089. doi:10.1136/bmj.h3089
19. Sackett DL, Rosenberg WM, Gray JA, Haynes RB, Richardson WS. Evidence based medicine: What it is and what it isn't. *BMJ*. 1996;312(7023):71–72. doi:10.1136/bmj.312.7023.71
20. Koca CG, Yildirim B, Bilgir E. Effects of bruxism on temporomandibular joint internal derangement in patients with unilateral temporomandibular joint pain: The role of magnetic resonance imaging diagnostics. *Cranio*. 2024;42(2):113–121. doi:10.1080/08869634.2021.1918959
21. Emshoff R, Brandlmaier I, Gerhard S, Strobl H, Bertram S, Rudisch A. Magnetic resonance imaging predictors of temporomandibular joint pain. *J Am Dent Assoc*. 2003;134(6):705–714. doi:10.14219/jada.archive.2003.0256
22. Manfredini D, Basso D, Arboretti R, Guarda-Nardini L. Association between magnetic resonance signs of temporomandibular joint effusion and disk displacement. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;107(2):266–271. doi:10.1016/j.tripleo.2008.03.033
23. Manfredini D, Arveda N, Guarda-Nardini L, Segù M, Collesano V. Distribution of diagnoses in a population of patients with temporomandibular disorders. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2012;114(5):e35–e41. doi:10.1016/j.oooo.2012.03.023
24. Manfredini D, Guarda-Nardini L, Winocur E, Piccotti F, Ahlberg J, Lobbezoo F. Research Diagnostic Criteria for Temporomandibular Disorders: A systematic review of axis I epidemiologic findings. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011;112(4):453–462. doi:10.1016/j.tripleo.2011.04.021
25. Tallents RH, Katzberg RW, Murphy W, Proskin H. Magnetic resonance imaging findings in asymptomatic volunteers and symptomatic patients with temporomandibular disorders. *J Prosthet Dent*. 1996;75(5):529–533. doi:10.1016/s0022-3913(96)90458-8
26. Takahara N, Nakagawa S, Sumikura K, Kabasawa Y, Sakamoto I, Harada H. Association of temporomandibular joint pain according to magnetic resonance imaging findings in temporomandibular disorder patients. *J Oral Maxillofac Surg*. 2017;75(9):1848–1855. doi:10.1016/j.joms.2017.03.026
27. Costa ALF, D'Abreu A, Cendes F. Temporomandibular joint internal derangement: Association with headache, joint effusion, bruxism, and joint pain. *J Contemp Dent Pract*. 2008;9(6):9–16. PMID:18784854.
28. de Souza Pinto GN, Grossmann E, Iwaki Filho L, et al. Correlation between joint effusion and morphology of the articular disc within the temporomandibular joint as viewed in the sagittal plane in patients with chronic disc displacement with reduction: A retrospective analytical study from magnetic resonance imaging. *Cranio*. 2021;39(2):119–124. doi:10.1080/08869634.2019.1582166
29. Görürgöz C, İçen M, Kurt MH, et al. Degenerative changes of the mandibular condyle in relation to the temporomandibular joint space, gender and age: A multicenter CBCT study. *Dent Med Probl*. 2023;60(1):127–135. doi:10.17219/dmp/147514
30. Campos MIG, Campos PSF, Cangussu MCT, Guimarães RC, Line SRP. Analysis of magnetic resonance imaging characteristics and pain in temporomandibular joints with and without degenerative changes of the condyle. *Int J Oral Maxillofac Surg*. 2008;37(6):529–534. doi:10.1016/j.ijom.2008.02.011
31. Cortés D, Exss E, Marholz C, Millas R, Moncada G. Association between disk position and degenerative bone changes of the temporomandibular joints: An imaging study in subjects with TMD. *Cranio*. 2011;29(2):117–126. doi:10.1179/crn.2011.020
32. Tanaka E, van Eijden T. Biomechanical behavior of the temporomandibular joint disc. *Crit Rev Oral Biol Med*. 2003;14(2):138–150. doi:10.1177/154411130301400207
33. de Leeuw R, Boering G, van der Kuijl B, Stegenga B. Hard and soft tissue imaging of the temporomandibular joint 30 years after diagnosis of osteoarthrosis and internal derangement. *J Oral Maxillofac Surg*. 1996;54(11):1270–1280; discussion 1280–1281. doi:10.1016/s0278-2391(96)90480-2

Association of periodontitis and gingivitis with stroke: A systematic review and meta-analysis

Meghna Dewan[1,A–F], Awadh Kishor Pandit[2,A–F], Lata Goyal[3,D–F]

[1] Private practice (former scientist at All India Institute of Medical Sciences (AIIMS), New Delhi, India)

[2] Department of Neurology, All India Institute of Medical Sciences (AIIMS), New Delhi, India

[3] Division of Periodontics, Department of Dentistry, All India Institute of Medical Sciences (AIIMS), Bhathinda, 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

Address for correspondence
Awadh Kishor Pandit
E-mail: akpandit.med@gmail.com

Abstract

Background. Stroke is among the leading causes of morbidity and mortality. Chronic inflammatory conditions may lead to atherosclerosis and a subsequent stroke.

Objectives. This systematic review and meta-analysis aimed to review the association of periodontitis and gingivitis with stroke.

Methods. An electronic search of PubMed, Ovid EMBASE, Ovid MEDLINE, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), Science Citation Index, Database of Abstracts and Reviews of Effects (DARE), and various clinical trial registries was conducted to include studies published up to February 2022. Data was retrieved by 2 independent reviewers. The Stata software, v. 13, was used to conduct a meta-analysis.

Results. Thirteen studies stated an association between periodontitis, determined based on clinical attachment loss (CAL), and stroke. Meanwhile, 6 studies described an association between gingivitis, determined based on the gingival index (GI), and stroke. Out of the 6 studies on gingivitis, 5 also investigated CAL, which means the meta-analysis included 14 studies in total. The total number of participants from the included studies was 35,937, and they were all above 17 years of age. There was a significant association between periodontitis and gingivitis and stroke and its all types. There was a significant association between periodontitis and stroke and its all types in 13 studies (*ES* (effect size): 1.32; 95% *CI* (confidence interval): 1.04–1.60), and between gingivitis and all stroke types in 6 studies (*ES*: 1.17; 95% *CI*: 0.42–1.92).

Conclusions. This systematic review indicated a significant association between stroke and periodontal disease in case–control, cohort and cross-sectional studies. The findings need to be further substantiated in prospective cohort studies with an optimal sample size.

Keywords: periodontitis, stroke, bacteria, cerebrovascular disorders, gingivitis and stroke

408

M. Dewan, A.K. Pandit, L. Goyal. Stroke and oral health

Introduction

Stroke is a major cause of disability and the third most common cause of mortality.[1] A "Lancet" editorial stated that "any stroke is a deplorable event, but a preventable stroke is a tragedy".[2] Furthermore, the prevalence of low-grade chronic inflammation in the adult population of industrialized countries is approx. 15–35%.[3]

There is evidence that chronic infectious diseases, including dental diseases, such as periodontitis and gingivitis, can lead to atherosclerosis, which increases the risk of stroke and coronary heart disease (CHD). In this regard, it is thought that the oral cavity acts as a perpetual source of infections and serves as a bacterial reservoir that augments systemic pathologies. Indeed, gingivitis and periodontitis are chronic inflammatory conditions of multifactorial origin that trigger a pro-inflammatory host immune response to bacterial products present in the oral cavity.

Studies on the mechanisms of periodontal and gingival inflammation suggest that microorganisms enter the bloodstream during chewing or tooth brushing via compromised tooth-supporting structures, such as the periodontal ligament and the alveolar bone, which leads to the formation of periodontal pockets or gingival recession, or both. Such microorganisms have been found in the carotid artery plaques and thrombectomy samples obtained from patients who suffered a stroke, implicating a probable association.[4] Although this association has been observed, it has certainly not been confirmed. Nonetheless, epidemiological studies (cohort, cross-sectional and case–control studies) have also demonstrated an association between periodontitis and gingivitis and a subsequent stroke of various types.[5,6] A previous systematic review and meta-analysis investigated the link between stroke and oral diseases, including periodontitis and gingivitis.[6] However, no studies have presented relevant clinical evidence regarding the abovementioned association, and there have been inconsistencies in the definitions of periodontitis and gingivitis. Therefore, the present study aimed to determine the association between the most common inflammatory conditions of the oral cavity – periodontitis and gingivitis – and stroke of various types.

Methodology

This systematic review and meta-analysis were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement.[7]

Search strategy

An electronic search of PubMed, Ovid EMBASE, Ovid MEDLINE, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), Science Citation Index, and Database of Abstracts and Reviews of Effects (DARE) was conducted to include articles published up to February 2022. Various clinical trial registries were also searched. All the articles retrieved during the search that studied the relationship between oral health and stroke were evaluated.

The literature search was restricted to English-language publications involving human subjects. The following combination of terms was searched: "stroke" OR "cerebrovascular disorders", (cerebrovascular disorders) AND (periodontitis), (cerebrovascular disorders) AND (gingivitis), (cerebrovascular accident) AND (gingivitis), (clinical attachment loss) AND (brain stroke), (periodontitis) AND (brain stroke), and (gingival inflammation) AND (stroke). The available secondary bibliographies were also reviewed, and a manual search of articles was undertaken as well.

The study is registered in the International Prospective Register of Systematic Reviews (PROSPERO) under No.: CRD42021237995.

Selection criteria

For inclusion in this systematic review and meta-analysis, studies had to be cohort (retrospective or prospective), cross-sectional or case–control in design, they had to include adult human subjects, and they had to define periodontitis in terms of clinical attachment loss (CAL) and gingivitis in terms of gingival index (GI).

Studies were excluded from further analysis if they did not report risk ratios (*RR*s) or odds ratios (*OR*s), or if they were published in a language other than English. Studies not addressing the association of periodontitis or gingivitis with various types of stroke were also excluded. If the study data was published in more than one publication, then the study published later or the publication with a more detailed report was included in the analysis.

Operational definitions

Periodontitis was defined as having CAL > 3 mm and more than 30% of sites involved. Periodontitis was expressed as CAL, or as CAL and probing depth (PD).[8]

The presence of gingivitis was defined as gingival inflammation measured using GI (Löe and Silness, 1963).

The stroke diagnosis was based on definitive examinations (i.e., an ischemic or hemorrhagic lesion on brain imaging and/or on clinical examination, with a definite neurological deficit).

Outcomes

The primary outcome was the association between periodontitis or gingivitis and strokes of various types, including an ischemic stroke, a hemorrhagic stroke and transient ischemic attacks (TIAs).

The secondary outcomes included tooth loss, bleeding on probing (BoP), PD, plaque index (PI), and gingival recession.

Data collection

Two reviewers (MD and AKP) retrieved data independently from the variety of sources outlined above. All of the included articles were scrutinized, and data on the study characteristics, methods, types of observations, patients, and outcomes was retrieved from texts, tables, figures, and graphs. Any disagreement between the 2 reviewers was resolved by consensus among the authors.

Quality assessment

The quality of the included studies was assessed using the Newcastle–Ottawa Scale (Tables 1,2).[9,10]

Table 1. Methodological quality of the included studies (case–control studies[5,13,15,16,18,19,24])

Study	Selection				Comparability	Exposure			Total score
	Is case definition adequate?	representativeness of cases	selection of controls	definition of controls	comparability of cases and controls based on the design or analysis	ascertainment of exposure	no-response rate	the same method of ascertainment for cases and controls	
Hashemipour et al. 2013[5]	*	*	*	*	0	**	*	*	8
Grau et al. 2004[13]	*	*	*	*	0	*	*	*	7
Pradeep et al. 2010[15]	*	0	0	*	0	*	*	*	5
Kim et al. 2010[16]	*	*	*	*	*	*	*	*	8
Diouf et al. 2015[18]	*	*	*	*	*	*	*	*	8
Leira et al. 2016[19]	*	*	*	*	*	*	*	*	8
Ghizoni et al. 2012[24]	*	0	0	*	*	*	*	*	6

Assessment: 4 stars for selecting participants and measuring exposure; 2 stars for comparability; and 3 stars for assessing the adequacy of the outcome and the follow-up. * score 1; no * score 0 (no description).

Table 2. Methodological quality of the included studies (cohort and cross-sectional studies[11,12,14,17,20–23])

Study	Selection				Comparability	Exposure			Total score
	representativeness of the exposed cohort	selection of the non-exposed cohort	ascertainment of exposure	outcome at the initiation of the study	comparability of the cohorts based on the design or analysis	ascertainment of the outcome	Was the follow-up long enough for the outcome to occur?	adequacy of the follow-up cohorts	
Loesche et al. 1998[11]	*	*	*	*	*	*	*	*	8
Elter et al. 2003[12]	*	*	*	*	*	*	*	*	8
Lee et al. 2006[14]	*	*	*	*	*	*	*	**	9
Sen et al. 2013[17]	*	*	*	*	*	*	*	*	8
Sen et al. 2018[20]	*	*	*	*	*	*	*	*	8
Beck et al. 2018[21]	*	*	*	*	*	*	*	*	8
Mascari et al. 2021[22]	*	*	**	*	*	*	*	*	9
Söder et al. 2015[23]	*	*	*	*	*	*	*	*	8

Assessment: 4 stars for selecting participants and measuring exposure; 2 stars for comparability; and 3 stars for assessing the adequacy of the outcome and the follow-up. * score 1; no * score 0 (no description).

Statistical analysis

Statistical analysis was performed using the Stata software, v. 13 (StataCorp, College Station, USA). Based on the data extracted from individual studies, the pooled estimates of effect size (*ES*) and 95% confidence intervals (*CI*s) were calculated for the association between periodontitis or gingivitis and stroke (ischemic, hemorrhagic or TIA). These were calculated based on various study designs and according to the reported *OR*, *RR* or hazard ratio (*HR*). The pooled estimates were presented as pooled *OR*, *RR* or *HR*.

The heterogeneity amongst studies was measured using the I^2 statistics. Heterogeneity was considered significant at $I^2 > 50\%$, in which case the random-effects model was used to synthesize the data; otherwise, the fixed-effects model was used for the estimation of the pooled *ES*. A *p*-value <0.05 was considered statistically significant.

Results

The comprehensive search generated 678 studies, and following a review of the titles, abstracts and full texts, 36 studies were included in the final analysis. A PRISMA flow diagram for the selected articles is shown in Fig. 1.

The total number of participants from the studies included in the meta-analysis was 35,937, with all subjects aged >17 years. Tables 3 and 4 provide a summary of the
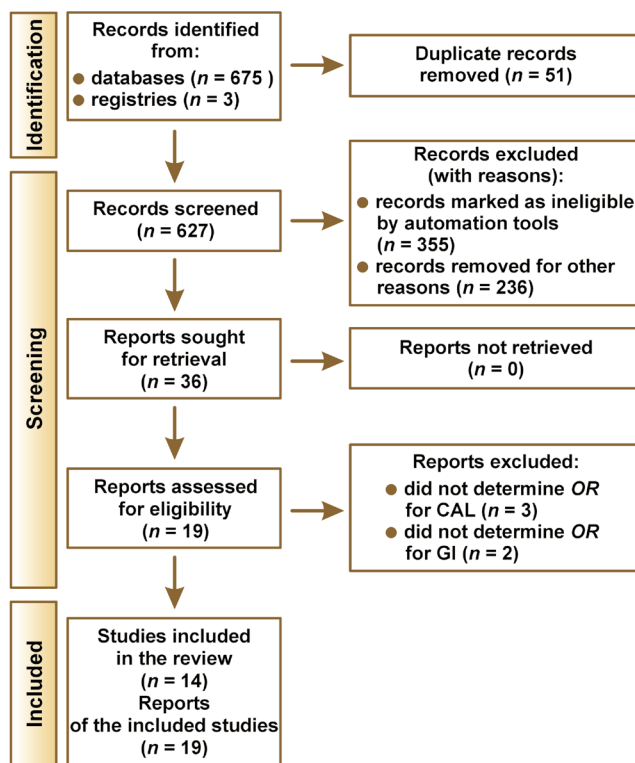
Fig. 1. Flow diagram of the study in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement[7]

OR – odds ratio; *CAL* – clinical attachment loss; *GI* – gingival index.

characteristics of the included studies. The secondary dental parameters from the included studies are shown in Table 5.

Outcome analysis

It was an inverse-variance random-effects meta-analysis.

Periodontitis and all stroke types

There was a significant association between periodontitis and stroke among patients from the various studies reviewed (*ES*: 1.32; 95% *CI*: 1.04–1.60). No significant heterogeneity was detected when reviewing the studies correlating periodontitis with stroke and its types; the inconsistency was moderate ($I^2 = 30.3\%$) (Fig. 2).

Gingivitis and all stroke types

There was a significant association between gingivitis and stroke among patients from the various studies reviewed (*ES*: 1.17; 95% *CI*: 0.42–1.92). There was no significant heterogeneity detected between the studies on gingivitis and stroke types; the inconsistency was moderate ($I^2 = 45.9\%$) (Fig. 3).
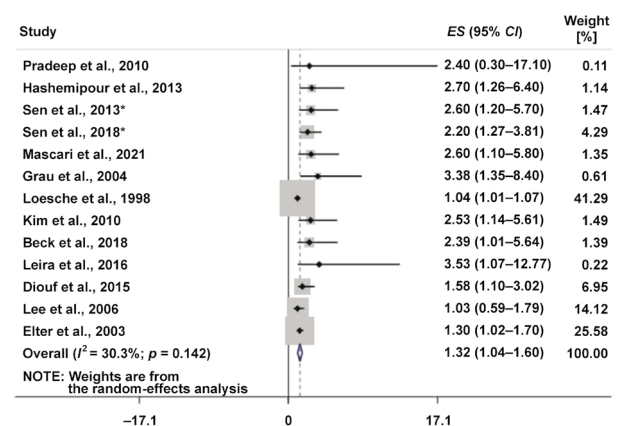
Fig. 2. Forest plots for periodontitis and stroke (all types) with the estimated risk (effect size (*ES*) (95% confidence interval (*CI*)) reported

* hazard ratio (*HR*) reported instead of *OR*.

Fig. 3. Forest plots for gingivitis and stroke (all types) with the estimated risk (effect size (*ES*) (95% confidence interval (*CI*)) reported

* *HR* reported instead of *OR*.

Table 3. General characteristics of the studies included in the systematic review (periodontitis – clinical attachment loss (CAL))

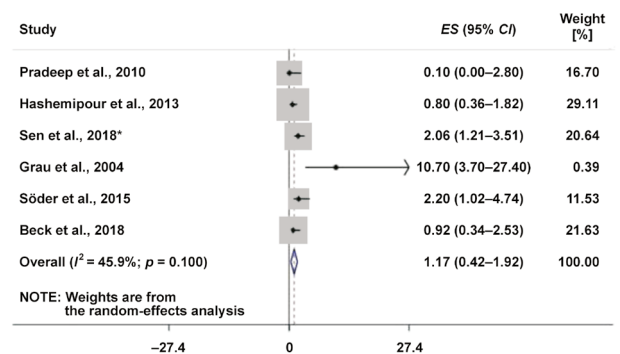| No.	Study	Country	Study design	Age [years]	Number of participants		Number of males	Stroke features	Dental features	Study key points	Study analysis
					controls	cases					
1	Hashemipour et al. 2013[5]	Iran	case–control study	52	100	100	86	ischemic stroke	CAL	age, gender, smoking, diabetes, renal failure, cholesterol, TG, HT, AF, peripheral cardiovascular disease, history of stroke, BoP, GI, CAL	there is a significant relation between stroke and periodontal disease
2	Loesche et al. 1998[11]	USA	cross-sectional study	<60	401	–	–	stroke	CAL	age, race, education, ever/current smoker, alcohol, number of medications, BMI, BP, dental examination	CAL > 6 mm was significantly associated with stroke
3	Elter et al. 2003[12]	USA	cross-sectional study	–	10,906	–	–	stroke/TIA	CAL	age, gender, race, demographics, lifestyle, education, income, Medicaid, smoking, diabetes, HT, CHD, edentulism, dental examination	CAL was found to be associated with stroke/TIA
4	Grau et al. 2004[13]	Germany	case–control study	18–75	168	300	–	stroke	CAL	age, female gender, school education, profession, smoking, alcohol, diabetes, hyperlipidemia, HT, AF, CAD, PAD, previous stroke/TIA, family history of stroke, dental examination	subjects with severe periodontitis (mean CAL = 6 mm) were at a 4.3 times higher risk of cerebral ischemia (95% CI: 1.85–10.2) than subjects with mild periodontitis (CAL = 3 mm) or without periodontitis
5	Lee et al. 2006[14]	USA	cross-sectional study	>60	5,123	–	–	stroke	CAL	age, gender, race, ethnicity, education, income, poverty, smoking, alcohol	there is evidence of an association between cumulative periodontal disease, based on PHS, and a history of stroke
6	Pradeep et al. 2010[15]	India	case–control study	54	100	100	–	CVA or stroke	CAL	age, gender, education, smoking, diet, diabetes, serum cholesterol, HT, cardiovascular disease, previous stroke, family history of stroke, PI, GI, CAL, PD	the mean CAL values for cases were significantly higher when compared with the control group (p < 0.05), which clearly shows that periodontitis may be a potential risk factor for stroke
7	Kim et al. 2010[16]	South Korea	case–control study	40–79	118	214	164	hemorrhagic stroke	CAL	age, male gender, duration of education ≥9 years, income ≥1,000 USD/month, smoking, alcohol, BMI ≥ 25, diabetes, HT, cardiac disease, family history of diabetes or cardiac disease, tooth brushing >2 times/day, annual dental visit, DMFT index, CAL	the association between periodontal disease (CAL > 6 mm) and hemorrhagic stroke was significant for males
8	Sen et al. 2013[17]	USA	cohort study	54.5	40	66	–	stroke/TIA	CAL	age, gender, race, education, income, ever/current smoker, BMI, diabetes, hyperlipidemia, hypercholesterolemia, HT, AF, CAD, TIA, PI, GI, CAL, PD, NIHSS, brain MRI/CT	HPD is associated with recurrent vascular events in stroke/TIA patients
9	Diouf et al. 2015[18]	Senegal	case–control study	–	120	120	–	stroke	CAL	sociodemographic characteristics, lifestyle behaviors, HT, general history, type of stroke (ischemic or hemorrhagic), PI, PBI, CAL, PD, CPITN	periodontal disease is associated with stroke in the Senegalese population
10	Leira et al. 2016[19]	Spain	case–control study	30–80	60	62	–	LI	CAL	age, male gender, smoking, alcohol, statins, diabetes, hypercholesterolemia, HT, PAD, IHD, FMPS, FMBS, periodontitis, CAL, PD, gingival recession, missing teeth, LI in the TOAST classification, MRI, CT, carotid Doppler	severe periodontitis is strongly associated with LI
11	Sen et al. 2018[20]	USA	cohort study	54.5	40	66	–	stroke/TIA	CAL	age, gender, race, education, income, ever/current smoker, BMI, diabetes, hyperlipidemia, hypercholesterolemia, HT, AF, CAD, TIA, PI, GI, CAL, PD, NIHSS, brain MRI/CT	HPD is associated with recurrent vascular events in stroke/TIA patients
12	Beck et al. 2018[21]	USA	original study	45–64	15,792	–	–	stroke	CAL	age, gender, race, education, never/former/current smoker, BMI, diabetes/non-diabetes, HT/non-HT, TC, periodontitis, BoP, PI, GI, CAL, tooth loss	periodontitis was found to be significantly associated with stroke
13	Mascari et al. 2021[22]	USA	cross-sectional study	64	265	–	–	stroke/TIA	CAL	age, gender, race, height, smoking, pre-diabetes, diabetes, hyperlipidemia, AF, CAD, history of TIA or stroke, HbA1c	a higher prevalence of stroke due to large-artery atherosclerosis was observed in patients with periodontal disease as compared to those without periodontal disease

TIA – transient ischemic attack; CVA – cerebrovascular accident; LI – lacunar infarct; TG – triglycerides; HT – hypertension; AF – atrial fibrillation; BoP – bleeding on probing; BMI – body mass index; BP – blood pressure; CHD – coronary heart disease; CAD – coronary artery disease, PAD – peripheral artery disease; PI – plaque index; PD – probing depth; DMFT index – decayed, missing and filled teeth (permanent teeth); NIHSS – National Institutes of Health Stroke Scale; MRI – magnetic resonance imaging; CT – computed tomography; CPITN – community periodontal index of treatment needs; IHD – ischemic heart disease; FMPS – full-mouth plaque score; FMBS – full-mouth bleeding score; TOAST – Trial of Org 10172 in Acute Stroke Treatment; TC – total; HbA1c – glycated hemoglobin; PHS – periodontal health status; HPD – high periodontal disease.

Table 4. General characteristics of the studies included in the systematic review (gingivitis – the gingival index (GI))

No.	Study	Country	Study design	Age [years]	Number of participants		Number of males	Stroke features	Dental features	Study key points	Study analysis
					controls	cases					
1	Hashemipour et al. 2013[5]	Iran	case–control study	52	100	100	86	ischemic stroke	CAL	age, gender, smoking, diabetes, renal failure, cholesterol, TG, HT, AF, peripheral cardiovascular disease, history of stroke, BoP, GI, CAL	there is a significant relation between stroke and periodontitis, but insignificant with gingivitis
2	Grau et al. 2004[13]	Germany	case–control study	18–75	168	300	–	stroke	CAL	age, female gender, school education, profession, smoking, alcohol, diabetes, hyperlipidemia, HT, AF, CAD, PAD, previous stroke/TIA, family history of stroke, dental examination	subjects with severe periodontitis (mean CAL = 6 mm) were at a 4.3 times higher risk of cerebral ischemia (95% CI: 1.85–10.2) than subjects with mild periodontitis (CAL = 3 mm) or without periodontitis
3	Pradeep et al. 2010[15]	India	case–control study	54	100	100	–	CVA or stroke	CAL	age, gender, education, smoking, diet, diabetes, serum cholesterol, HT, cardiovascular disease, previous stroke, family history of stroke, PI, GI, CAL, PD	the mean CAL values for cases were significantly higher when compared with the control group (p < 0.05), which clearly shows that periodontitis may be a potential risk factor for stroke
4	Sen et al. 2018[20]	USA	cohort study	54.5	40	66	–	stroke/TIA	CAL	age, gender, race, education, income, ever/current smoker, BMI, diabetes, hyperlipidemia, hypercholesterolemia, HT, AF, CAD, TIA, PI, GI, CAL, PD, NIHSS, brain MRI/CT	HPD is associated with recurrent vascular events in stroke/TIA patients
5	Beck et al. 2018[21]	USA	original study	45–64	15,792	–	–	stroke	CAL	age, gender, race, education, never/former/current smoker, BMI, diabetes/non-diabetes, HT/non-HT, TC, periodontitis, BoP, PI, GI, CAL, tooth loss	periodontitis was found to be significantly associated with stroke
6	Söder et al. 2015[23]	Finland	cohort study	30–40	39	1,637	838	stroke	GI	age, gender, education, income, smoking, CI, PI, GI, number of missing teeth	GI appeared to be a principal independent predictor associated with a higher risk of stroke

CI – calculus index.

Discussion

This systematic review aimed to find an association between various types of stroke and periodontitis or gingivitis. A total of 19 studies were included in the analysis, of which 13 studies were on the association of periodontitis with stroke types, and 6 were on the association between gingivitis and stroke. The results indicated an association between stroke and both periodontitis and gingivitis, with the pooled results of *ES*: 1.32; 95% *CI*: 1.04–1.60 and *ES*: 1.17; 95% *CI*: 0.42–1.92, respectively.

Out of the 13 studies on periodontitis, 4 were cross-sectional studies, 2 were cohort studies, 1 was an original study, and the remaining 6 were case–control studies. A significant association between periodontitis and stroke (all types) was found in all case–control studies. Cross-sectional and cohort studies also showed that periodontitis was linked with various types of stroke. The association between periodontitis and stroke has been studied previously, but the criteria for the evaluation of periodontitis varied across studies, which limited the generalization of study findings. Indeed, significant heterogeneity was noted among such studies, meaning that the association was not robust. This may have been due to the methodological quality of the studies. In the current systematic review, periodontitis was defined in terms of CAL, which is a better parameter for diagnosing periodontitis than either PD or alveolar bone loss.

Several studies included in this meta-analysis had limitations in terms of methodological quality, which impeded the observations of the study sample, the quality of measurements as well as blinding. In 3 of the case–control studies – by Kim et al.,[16] Diouf et al.[18] and Ghizoni et al.[24] (the study was excluded from the meta-analysis) – the misclassification bias was observed. Non-differential misclassification usually dilutes the estimate of an association, as exposure is unrelated to other variables. In such a study, the link between the periodontal status and the risk of stroke is likely to be underestimated, although overestimation is not entirely impossible. In the study by Pradeep et al., the selection bias was detected in the control subjects.[15] The study enrolled persons who had been admitted to the same department, as they were suffering from neurological diseases, such as degenerative disease, myasthenia gravis and Wilson's disease.[15] The selection bias was also detected in the study by Leira et al., along with a lack of validity of the patient history, which was assessed by interviewing the participants.[19] The selection bias was also observed in the study by Mascari et al.[22] A small sample size was observed in the study by Sen et al.[17]

Six studies explored the association between GI and stroke. One study was an original study, 2 studies were cohort, and 3 were case–control studies. No association

Table 5. Secondary dental features in the included studies

No.	Study	Country	Study design	Stroke features	Secondary dental features	Study analysis
1	Loesche et al. 1998[11]	USA	cross-sectional study	stroke	DMFT index, PD, gingival recession, presence of dentures and other prosthetic devices	PI and the oral hygiene habits relating to brushing, flossing and the frequency of having teeth cleaned by a dentist/hygienist were significantly associated with CVA
2	Elter et al. 2003[12]	USA	cross-sectional study	stroke/TIA	edentulism	edentulism was found to be associated with stroke/TIA
3	Grau et al. 2004[13]	Germany	case–control study	stroke	dental visit <1 time/year, DMFT index, PI, bone loss	subjects with severe periodontitis (mean CAL = 6 mm) had an increased risk of cerebral ischemia; gingivitis and bone loss were found to have a strong independent association with stroke; caries, the number of missing teeth and PI were not independent risk factors
4	Lee et al. 2006[14]	USA	cross-sectional study	stroke	PHS	no statistically significant association was found between PHS and stroke
5	Pradeep et al. 2010[15]	India	case–control study	CVA or stroke	PI, PD	PD > 4.5 mm was found to be the most significant risk factor for stroke; the mean PI value was also found to be significant
6	Kim et al. 2010[16]	South Korea	case–control study	hemorrhagic stroke	tooth brushing >2 times/day, annual dental visit >1, DMFT index, number of missing teeth	stroke was not associated with the number of missing teeth, the experience of dental caries or the annual dentist visits
7	Sen et al. 2013[17]	USA	cohort study	stroke/TIA	PI, PD, tooth loss	HPD is associated with recurrent vascular events in stroke/TIA patients; no association was observed between GI and stroke; regular dental care was associated with a lower adjusted stroke risk
8	Diouf et al. 2015[18]	Senegal	case–control study	stroke	PI, PBI, PD, CPITN	periodontal disease and various periodontal parameters were significantly associated with stroke
9	Leira et al. 2016[19]	Spain	case–control study	LI	PI, PBI, PD, CPITN	FMPS, FMBS, PD, gingival recession, and missing teeth were significantly associated with LI
10	Sen et al. 2018[20]	USA	cohort study	stroke/TIA	PI, tooth loss	HPD is associated with recurrent vascular events in stroke/TIA patients
11	Beck et al. 2018[21]	USA	original study	stroke	BoP, PI, tooth loss	high gingival inflammation, tooth loss and severe tooth loss were significantly associated with diabetes, CHD, hs-CRP, and IL-6
12	Mascari et al. 2021[22]	USA	cross-sectional study	stroke/TIA	PD, gingival margin position	a higher prevalence of stroke due to large-artery atherosclerosis was observed in patients with periodontal disease as compared to those without periodontal disease
13	Söder et al. 2015[23]	Finland	cohort study	stroke	number of dental visits, CI, PI, GI, number of missing teeth	CI and GI were significantly higher in the stroke group
14	Ghizoni et al. 2012[24]	Brazil	case–control study	ischemic/hemorrhagic stroke	BoP, PI, CAL, PD, number of missing teeth, Pg and Aa bacteria; age- and gender-matched	stroke patients had deeper pockets, and presented with increased BoP and PI values; in their pockets, increased amounts of Pg harbored

Pg – *Porphyromonas gingivalis*; Aa – *Aggregatibacter actinomycetemcomitans*; hs-CRP – high-sensitivity C-reactive protein; IL-6 – interleukin 6.

was found between gingival inflammation and the incidence of stroke in the studies by Hashemipour et al.[5] and Sen et al.[20] Meanwhile, the other 4 studies found that high gingival inflammation was associated with stroke, and concluded that gingivitis was a risk factor for various types of stroke.

Secondary outcomes studied were tooth loss, BoP, PD, PI, and gingival recession. Most studies found that increased PD and tooth loss were independent risk factors for a stroke event. Söder et al. concluded that the calculus index (CI) and GI were significantly associated with stroke.[23] Beck et al. studied cytokine levels and found C-reactive protein (CRP) and interleukin 6 (IL-6) to be higher in patients with a cardiovascular risk.[21]

The quality assessment of the cohort studies with the Newcastle–Ottawa Scale indicated that the studies provided moderate to good quality evidence. Amongst the various cohort studies, it was found that information on some other risk factors for stroke, such as body mass index (BMI), a family history and smoking, as well as some co-morbidities, e.g., inflammatory diseases,[12] was not collected. A weakness was the lack of data on certain stroke risk factors, such as hypertension, diabetes and dyslipidemia, which were not available for analysis due to the nature of the study. Furthermore, data from the oral microbiomes of subjects was not included in the study by Söder et al.[23] Some studies included in the analysis had small sample sizes,[20,23] so in those cases, drawing any conclusions may be biased.

Gingivitis and periodontitis are chronic inflammatory conditions that can result from a pro-inflammatory host immune response in reaction to pathogenic bacteria. These microorganisms enter the bloodstream during mastication, tooth brushing and minor dental procedures, resulting in transient bacteremia. Pro-inflammatory cytokines, such as IL-6 and interferons, are produced in response to endothelial activation, which is responsible for plaque rupture, platelet aggregation, thrombus formation, and thromboembolism, all of which can lead to stroke.

In a systematic review by Fernandes Fagundes et al., it was concluded that periodontitis was a significant risk factor for stroke.[6] However, there was no clear-cut definition of periodontitis, and gingival inflammation was not taken into consideration.[6] In another systematic review by Pillai et al., the relationship between oral health and stroke was studied.[25] The main aim of the study was to find a relationship between stroke and oral health. It was concluded that stroke patients had poor hygiene practices, but there was no conclusive evidence regarding gingivitis. Dental prophylaxis and various dental procedures reduced the incidence of stroke.[25]

In a systematic review, Scannapieco et al. explored the link between periodontitis, atherosclerosis, cardiovascular disease, and stroke.[26] They concluded that a lack of uniform definitions and periodontal diagnosis measures complicated the interpretation of data and results, with some studies showing a moderate association and others showing no association at all.[26]

Larvin et al. found a modest but increased risk of cardiovascular disease in periodontal patients.[27] All of the possible outcomes of cardiovascular events were measured, including infarction, coronary artery disease (CAD) and stroke, while the diagnosis of periodontal disease was both self-reported and clinically confirmed.[27]

Oral disease (gingivitis and periodontitis) has been implicated as a factor in an increased incidence of stroke. However, whether oral disease can modify stroke through the role of inflammation in atherogenesis and the physiopathology of cerebral ischemia is not clear. Many long-term studies are being conducted in this area, with some proven and unproven data available. Therefore, a consistent relationship exists between periodontal disease and stroke in terms of biological and dental knowledge, and it is appropriate to analyze each case individually.[28]

One case–control study by Ghizoni et al.[24] was included in this systematic review, but was later excluded from the meta-analysis, since the *CI* was too wide.

Future directions

The proposed future studies need to confirm the association between periodontitis and gingivitis and stroke/TIA.

The proposed study design should be a prospective cohort study. The following selection, comparability and outcome criteria should be considered:
– selection:
  * representativeness of the exposed cohort,
  * selection of the non-exposed cohort,
  * ascertainment of exposure – 2 dentists should evaluate the dental condition, and the inter-observer agreement should be targeted at >0.8,
  * outcome at the initiation of study – ensure there is no stroke/TIA at the time of enrollment,
  * confounders to be included – all the known prognostic factors for stroke should be included in the study, with validated criteria;
– comparability – comparability of the cohorts based on the design or analysis;
– outcome:
  * ascertainment of the outcome – clinico-radiological confirmation of the diagnosis of stroke/TIA,
  * follow-up – at least 10 years, or more, of follow-up to ascertain a definite association between the dental condition and stroke,
  * adequacy of the follow-up cohorts – minimal attrition of the participants from the study.
Cases to be included – patients with chronic periodontitis and gingivitis. The cases should not have evidence of other systemic illnesses or inflammatory diseases.

The microbiological evaluation of dental infection/inflammation leading to atherosclerosis and a subsequent stroke should be confirmed after the exclusion of all possible confounders. The analysis should include healthy individuals, who need to be followed up once dental infection/inflammation develops and a subsequent stroke/TIA occurs.

Conclusions

This systematic review indicated a significant association between stroke and periodontal disease in case–control, cohort and cross-sectional studies.

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.

ORCID iDs

Meghna Dewan https://orcid.org/0000-0003-4678-0260
Awadh Kishor Pandit https://orcid.org/0000-0003-2847-9730
Lata Goyal https://orcid.org/0000-0002-0385-1316

References

1. Kamalakannan S, Gudlavalleti AS, Murthy Gudlavalleti VS, Goenka S, Kuper H. Incidence & prevalence of stroke in India: A systematic review. *Indian J Med Res*. 2017;146(2):175–185. doi:10.4103/ijmr.IJMR_516_15
2. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet*. 2005;366(9499):1809–1820. doi:10.1016/S0140-6736(05)67728-8
3. Joshipura K. The relationship between oral conditions and ischemic stroke and peripheral vascular disease. *J Am Dent Assoc*. 2002;133(Suppl):23S–30S. doi:10.14219/jada.archive.2002.0373
4. Patrakka O, Pienimäki JP, Tuomisto S, et al. Oral bacterial signatures in cerebral thrombi of patients with acute ischemic stroke treated with thrombectomy. *J Am Heart Assoc*. 2019;8(11):e012330. doi:10.1161/JAHA.119.012330
5. Hashemipour MA, Afshar AJ, Borna R, Seddighi B, Motamedi A. Gingivitis and periodontitis as a risk factor for stroke: A case–control study in the Iranian population. *Dent Res J (Isfahan)*. 2013;10(5):613–619. PMID:24348618. PMCID:PMC3858735.
6. Fernandes Fagundes NC, Carvalho Almeida AP, Barbosa Vilhena KF, Magno MB, Maia LC, Lima RR. Periodontitis as a risk factor for stroke: A systematic review and meta-analysis. *Vasc Health Risk Manag*. 2019;5:519–532. doi:10.2147/VHRM.S204097
7. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71
8. Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. *J Periodontol*. 2018;89(Suppl 1):S159–S172. doi:10.1002/JPER.18-0006
9. Higgins JP, Green S, eds. *Cochrane Handbook for Systematic Reviews of Interventions*, v. 5.1.0 (updated March 2011). https://handbook-5-1.cochrane.org/. Accessed April 1, 2022.
10. Wells GA, Shea B, O'Connell D, et al. The Newcastle–Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed April 1, 2022.
11. Loesche WJ, Schork A, Terpenning MS, Chen YM, Kerr C, Dominguez BL. The relationship between dental disease and cerebral vascular accident in elderly United States veterans. *Ann Periodontol*. 1998;3(1):161–174. doi:10.1902/annals.1998.3.1.161
12. Elter JR, Offenbacher S, Toole JF, Beck JD. Relationship of periodontal disease and edentulism to stroke/TIA. *J Dent Res*. 2003;82(12):998–1001. doi:10.1177/154405910308201212
13. Grau AJ, Becher H, Ziegler CM, et al. Periodontal disease as a risk factor for ischemic stroke. *Stroke*. 2004;35(2):496–501. doi:10.1161/01.STR.0000110789.20526.9D
14. Lee HJ, Garcia RI, Janket SJ, et al. The association between cumulative periodontal disease and stroke history in older adults. *J Periodontol*. 2006;77(10):1744–1754. doi:10.1902/jop.2006.050339
15. Pradeep AR, Hadge P, Raju PA, Shetty SR, Shareef K, Guruprasad CN. Periodontitis as a risk factor for cerebrovascular accident: A case–control study in the Indian population. *J Periodontal Res*. 2010;45(2):223–228. doi:10.1111/j.1600-0765.2009.01220.x
16. Kim HD, Sim SJ, Moon JY, Hong YC, Han DH. Association between periodontitis and hemorrhagic stroke among Koreans: A case–control study. *J Periodontol*. 2010;81(5):658–665. doi:10.1902/jop.2010.090614
17. Sen S, Sumner R, Hardin J, et al. Periodontal disease and recurrent vascular events in stroke/transient ischemic attack patients. *J Stroke Cerebrovasc Dis*. 2013;22(8):1420–1427. doi:10.1016/j.jstrokecerebrovasdis.2013.06.024
18. Diouf M, Basse A, Ndiaye M, Cisse D, Lo CM, Faye D. Stroke and periodontal disease in Senegal: Case–control study. *Public Health*. 2015;129(12):1669–1673. doi:10.1016/j.puhe.2015.02.033
19. Leira Y, López-Dequidt I, Arias S, et al. Chronic periodontitis is associated with lacunar infarct: A case–control study. *Eur J Neurol*. 2016;23(10):1572–1579. doi:10.1111/ene.13080
20. Sen S, Giamberardino LD, Moss K, et al. Periodontal disease, regular dental care use, and incident ischemic stroke. *Stroke*. 2018;49(2):355–362. doi:10.1161/STROKEAHA.117.018990
21. Beck JD, Moss KL, Morelli T, Offenbacher S. Periodontal profile class is associated with prevalent diabetes, coronary heart disease, stroke, and systemic markers of C-reactive protein and interleukin-6. *J Periodontol*. 2018;89(2):157–165. doi:10.1002/JPER.17-0426
22. Mascari R, Vezzeti A, Orofino C, et al. Periodontal disease association with large-artery atherosclerotic stroke. *J Neurol Disord Stroke*. 2021;8(1):1173. PMID:35243483. PMCID:PMC8889900.
23. Söder B, Meurman JH, Söder PÖ. Gingival inflammation associates with stroke – a role for oral health personnel in prevention: A database study. *PLoS One*. 2015;10(9):e0137142. doi:10.1371/journal.pone.0137142
24. Ghizoni JS, de Assis Taveira LA, Garlet GP, et al. Increased levels of *Porphyromonas gingivalis* are associated with ischemic and hemorrhagic cerebrovascular disease in humans: An in vivo study. *J Appl Oral Sci*. 2012;20(1):104–112. doi:10.1590/s1678-77572012000100019
25. Pillai RS, Iyer K, Spin-Neto R, Kothari SF, Nielsen JF, Kothari M. Oral health and brain injury: Causal or casual relation? *Cerebrovasc Dis Extra*. 2018;8(1):1–15. doi:10.1159/000484989
26. Scannapieco FA, Bush RB, Paju S. Associations between periodontal disease and risk for nosocomial bacterial pneumonia and chronic obstructive pulmonary disease. A systematic review. *Ann Periodontol*. 2013;8(1):54–69. doi:10.1902/annals.2003.8.1.54
27. Larvin H, Kang J, Aggarwal VR, Pavitt S, Wu J. Multimorbid disease trajectories for people with periodontitis. *J Clin Periodontol*. 2021;48(12):1587–1596. doi:10.1111/jcpe.13536
28. Dhadse P, Gattani D, Mishra R. The link between periodontal disease and cardiovascular disease: How far we have come in last two decades? *J Indian Soc Periodontol*. 2010;14(3):148–154. doi:10.4103/0972-124X.75908

Resistance of CAD/CAM composite resin and ceramic occlusal veneers to fatigue and fracture in worn posterior teeth: A systematic review

Karelys Del Cisne Maldonado[1,A–D], Juan Andres Espinoza[1,2,A–E], Daniela Andrea Astudillo[3,A–C,F], Bolivar Andres Delgado[4,A–C,F], Wilson Daniel Bravo[3,A–C,F]

[1] Graduate Program in Oral Rehabilitation and Implant-Assisted Prosthesis, University of Cuenca, Ecuador

[2] Investigation Department, University of Cuenca, Ecuador

[3] Graduate Department of Oral Rehabilitation and Implant-Assisted Prosthesis, University of Cuenca, Ecuador

[4] Department of Prosthodontics, Catholic University of Cuenca, Ecuador

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

Abstract

Severe tooth wear is related to substantial loss of tooth structure, with dentin exposure and significant loss ($\geq 1/3$) of the clinical crown. The objective of this systematic review was to summarize and analyze the scientific evidence regarding the mechanical performance of computer-aided design/computer-aided manufacturing (CAD/CAM) composite resin and CAD/CAM lithium disilicate ceramic occlusal veneers, in terms of fatigue and fracture resistance, on severely worn posterior teeth. Currently, occlusal veneers are an alternative for treating worn posterior teeth. Although scientific evidence demonstrates the good performance of lithium disilicate occlusal veneers, there are less brittle materials with a modulus of elasticity more similar to dentin than ceramics, such as resin CAD/CAM blocks. Therefore, it is important to identify which type of material is best for restoring teeth with occlusal wear defects and which material can provide better clinical performance. This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive search of the PubMed, Embase, Web of Science, Scopus, Cochrane, OpenGrey, Redalyc, DSpace, and Grey Literature Report databases was conducted and supplemented by a manual search, with no time or language limitations, until January 2022. We aimed to identify studies evaluating the fatigue and fracture resistance of CAD/CAM composite resin and ceramic occlusal veneers. The quality of the full-text articles was evaluated according to the modified Consolidated Standards of Reporting Trials (CONSORT) criteria for in vitro studies, and 400 articles were initially identified. After removing duplicates and applying the selection criteria, 6 studies were included in the review. The results demonstrated that the mechanical performance of CAD/CAM composite resin occlusal veneers is comparable to that of CAD/CAM lithium disilicate occlusal veneers in terms of fatigue and fracture resistance.

Keywords: tooth wear, ceramics, survival rate, composite resin, CAD/CAM

Introduction

In the first clinical study measuring tooth wear in young patients, the authors observed a mean annual occlusal enamel wear of 29 µm in molars and 15 µm in premolars.[1] A 2015 report estimated that the mean height of the maxillary incisor crown at 10 years of age was 11.94 mm, and decreased to 10.93 mm in 70-year-old patients, corresponding to a loss of 1.01 mm (1,010 µm) in 60 years.[2] The wear was greater in the mandibular incisors, with the mean crown height at 10 years of age being 9.58 mm, which decreased to 8.12 mm in patients aged 70 years old, resulting in a loss of 1.46 mm (1,460 µm) over the course of 60 years. These values correspond to a physiological annual wear rate of 16.8 µm for the maxillary incisors and 24.3 µm for the mandibular incisors.[2]

It is important to differentiate between severe tooth wear and pathological tooth wear. The latter refers to atypical tooth wear for the patient's age that causes pain or discomfort, functional problems, or deterioration of the aesthetic appearance which, if progresses, can lead to undesirable complications of increasing complexity.[2] Severe tooth wear is related to substantial loss of tooth structure, with dentin exposure and significant loss ($\geq 1/3$) of the clinical crown.[3] However, not all cases of severe tooth wear can be considered pathological, especially among elderly people. According to an epidemiological study conducted in 2015, the estimated prevalence of erosive tooth wear in children and adolescents was 30.4%.[4] The most recent European consensus on the management of severe tooth wear[3] recommends the use of indices such as the Basic Erosive Wear Examination (BEWE)[5] and the Tooth Wear Evaluation System (TWES)[6] for diagnosis. Severe tooth wear can be attributed to a number of factors, including excessive consumption of carbonated beverages, a high-acid diet, gastric diseases, anorexia, bulimia, teeth grinding, and the use of highly abrasive pastes.[7–12] These factors can affect the patient in several ways, including the loss of vertical dimension, sensitivity due to dentin exposure, poor aesthetics, and neuromuscular disorders.[7,11,12]

Restorative alternatives have been sought to solve these problems, such as the placement of metal-free crowns. Although this technique has shown a high survival rate (92% at 5 years and 85.5% at 10 years),[13] it requires mechanical retention, necessitating the removal of more dental tissue, including healthy tissue. Advances in dental materials and adhesive techniques have led to a reduction in the indications for crowns.[14,15] Occlusal veneers have emerged as a viable alternative for the treatment of posterior tooth wear, as they require minimal tooth preparation, ranging from 0.4 mm to 0.6 mm at the level of the developmental groove and from 1 mm to 1.3 mm at the tip of the cusp, largely preserving healthy dental tissue. Due to the bonding characteristics of these materials and the more intuitive preparation guided by anatomical considerations, there are instances where no dental tissue is removed.[16–18]

Advances in computer-aided design/computer-aided manufacturing (CAD/CAM) technology and bonding procedures (immediate dentin sealing)[19,20] have enabled the fabrication of thin occlusal veneers without compromising their performance.[16] Scientific evidence indicates that lithium disilicate occlusal veneers exhibit excellent performance.[21,22] However, less brittle materials with a modulus of elasticity comparable to that of dentin, such as composite resin, are also available.[17,19,23] A number of studies, the majority of which were conducted in a laboratory setting, have evaluated the mechanical properties of occlusal veneers using universal test machines and mastication simulators under physiological and/or pathological occlusal loading conditions.[19,23–25] However, there is no up-to-date systematic review that allows the clinician to make an informed decision regarding the most appropriate material for restoring teeth with occlusal wear. Therefore, the objective of this systematic review is to analyze and summarize the scientific evidence evaluating the mechanical performance of CAD/CAM composite resin and lithium disilicate ceramic occlusal veneers in severely worn posterior teeth, with a particular focus on the fatigue and fracture resistance.

Material and methods

Registration protocol

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[26] Additionally, the review was registered in the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) (doi:10.37766/inplasy2021.10.0036; https://inplasy.com/inplasy-2021-10-0036).

Search strategy

The purpose of the search was to address the following question: in posterior teeth with severe tooth wear, can occlusal veneers made from CAD/CAM composite resin blocks perform better mechanically in terms of fatigue and fracture resistance compared to CAD/CAM lithium disilicate occlusal veneers? The research question was developed in accordance with the Population, Intervention, Comparison, and Outcome (PICO) framework.

Three independent researchers (KM, JE and DA) conducted an exhaustive electronic search of the following databases: PubMed, Scopus, Cochrane, Embase, and Web of Science, to identify relevant articles published before January 2022. The Medical Subject Headings (MeSH), Embase subject headings (Emtree) and free terms were used without restrictions in terms of language and year of publication. A search strategy is presented in the supplementary materials (available on request from the

corresponding author). To identify other potentially relevant articles, 2 researchers (KM and JE) conducted a manual search of the bibliographic citations of the included articles and the following journals: Dental Materials; Journal of Dental Restoration; Journal of Dentistry; Journal of Oral Rehabilitation; Journal of Esthetic and Restorative Dentistry; Dental Materials Journal; Journal of Material Sciences. The search for grey literature was performed by KM and JE in the OpenGrey, Redalyc, DSpace, and Grey Literature Report databases.

Eligibility criteria

The present systematic review included studies on the indirect restoration of worn posterior teeth with machined materials. The studies compared the mechanical properties of CAD/CAM composite resin and ceramic materials, including the fatigue and fracture resistance. This review included randomized controlled trials, non-randomized controlled trials and in vitro studies.

Studies investigating CAD/CAM restorations on endodontically treated teeth, as well as crown, inlay, onlay, and implant restorations, case reports, literature reviews, expert opinions, and systematic reviews were excluded.

Screening and selection

Two researchers (KM and JE) independently selected the studies for inclusion based on their titles and abstracts. If a decision regarding inclusion could not be made because of insufficient data in the title and abstract, the complete manuscript was obtained for further analysis. The articles in which both researchers concurred were selected. The articles selected for full-text reading were evaluated independently by 2 researchers (KM and JE). Any disagreement regarding the eligibility of the included studies was resolved through discussion and consensus, or by a third reviewer (DA). Only papers that met all the eligibility criteria were included.

The modified Consolidated Standards of Reporting Trials (CONSORT) tool[27] was employed to assess the methodological quality of the articles included in the study in terms of their correct implementation and the structure of the abstract, introduction, methods, results, discussion, and funding.

Data extraction

A data extraction protocol was defined and evaluated by 2 authors (KM and JE). The data was extracted independently from the full-text articles using a standardized form in electronic format (Microsoft Excel 2016; Microsoft Corporation, Redmond, USA). The information was classified according to the authors, year of the study, study design, type of material, sample size, objectives, testing machine used, and conclusions (Table 1).

Risk of bias assessment

Two authors (KM and JE) independently evaluated the risk of bias in the studies included in this review based on a previous study.[28] The following parameters were assessed: tooth randomization, the use of teeth free of caries or restorations, the use of materials following manufacturers' instructions, the use of teeth with similar dimensions, tooth preparation by the same operator, the description of sample size calculations, and blinding of the testing machine operator. If the author reported the parameter, the article received a "yes" (Y) for that specific parameter; if the information was not found, the article received a "no" (N). Articles reporting 1 to 3 items were classified as exhibiting a high risk of bias, 4 or 5 items as a medium risk of bias, and 6 or 7 items as a low risk of bias. Any disagreements regarding the risk of bias were resolved through consensus. If a consensus could not be reached, the third author (DA) intervened.

Results

Selection of studies

A PRISMA flowchart, which provides a summary of the selection process, is presented in Fig. 1. A total of 400 studies were identified through the search process, with 25 duplicate records being removed. Another 4 studies were
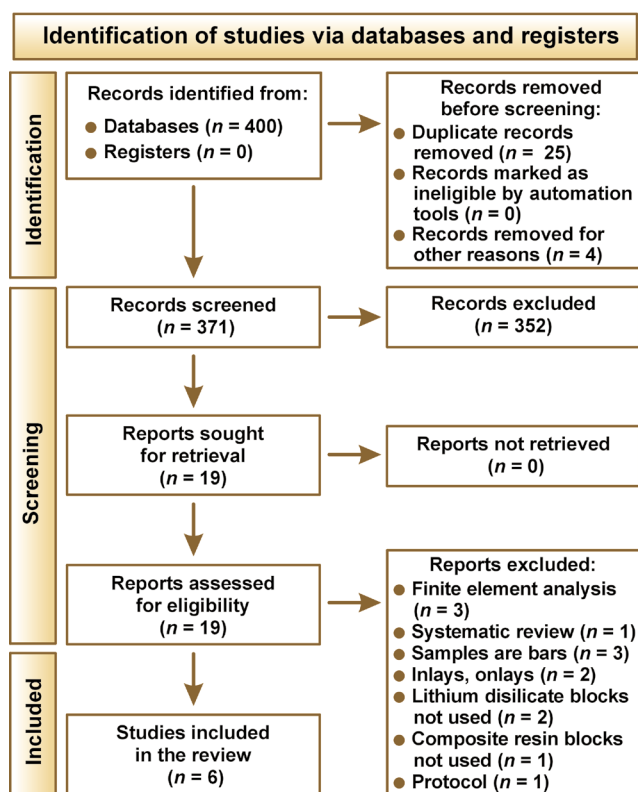

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram

Table 1. Summary of the studies included in the systematic review

Study	Study design	Material	Sample size	Objectives	Testing machine	Conclusions
Andrade et al. 2018[23]	in vitro	• IPS e.max CAD • VITA ENAMIC® • Lava™ Ultimate	70 human third molars	To evaluate, in vitro, the influence of CAD/CAM restorative materials and their thickness (0.6 mm and 1.5 mm) on the fracture resistance of teeth restored with occlusal veneers.	• cyclic mechanical loading: ER-11000 (ERIOS, São Paulo, Brazil) • fracture resistance testing: EMIC DL-2000 (EMIC, São José dos Pinhais, Brazil)	The occlusal veneers exhibited the fracture resistance similar to that of sound teeth.
Al-Akhali et al. 2017[25]	in vitro	• IPS e.max CAD • VITA ENAMIC®	64 human maxillary first premolars	To evaluate the influence of thermodynamic loading on the durability and fracture resistance behavior of occlusal veneers fabricated from different dental CAD/CAM materials.	• cyclic loading fatigue: dual-axis computerized chewing simulator (Willytec, Feldkirchen-Westerham, Germany) • fracture resistance testing: Zwick Z010/TN2A (Zwick GmbH, Ulm, Germany)	The materials tested may be considered a viable treatment for the restoration of occlusal surfaces of posterior teeth.
Al-Akhali et al. 2019[29]	in vitro	• IPS e.max CAD • VITA ENAMIC®	64 human maxillary first premolars	To evaluate the influence of thermomechanical fatigue loading on the fracture strength of minimally invasive occlusal veneer restorations fabricated from different CAD/CAM materials and bonded to human maxillary premolars using the self-etching bonding technique.	• cyclic loading fatigue: dual-axis computerized chewing simulator (Willytec) • fracture resistance testing: Zwick Z010/TN2A (Zwick GmbH)	Thermomechanical fatigue decreased the survival rate and fracture strength of all tested CAD/CAM materials when bonded to enamel using the self-etching technique.
Heck et al. 2019[30]	in vitro	• IPS e.max CAD • Lava™ Ultimate	84 human molars	To determine whether ceramics or nanoceramic composites with an ultrathin layer thickness of 0.3–0.5 mm could be used to restore pressure-loaded occlusal dentin and enamel defects.	• fatigue simulations: computer-controlled chewing simulator (MUC 2; Willytec GmbH, Gräfelfing, Germany)	The tested occlusal veneers are recommended for the treatment of occlusal tooth loss with ultrathin veneers.
Magne et al. 2010[19]	in vitro	• IPS e.max CAD • Paradigm™ MZ100	30 human maxillary molars	To assess and compare the fatigue resistance of composite resin and ceramic posterior occlusal veneers.	• fatigue testing: closed-loop servohydraulics (Mini Bionix II; MTS Systems Corp., Eden Prairie, USA)	CAD/CAM composite resin posterior occlusal veneers had a significantly higher fatigue resistance when compared to the ceramic veneers.
Schlichting et al. 2011[17]	in vitro	• IPS e.max CAD • Paradigm™ MZ100 • XR experimental blocks (reinforced with short polyethylene fibers)	40 human maxillary molars	To assess the influence of CAD/CAM restorative materials (ceramic vs. composite resin) on the fatigue resistance of ultra-thin occlusal veneers.	• fatigue testing: closed-loop servohydraulics (Mini Bionix II; MTS Systems Corp.)	CAD/CAM composite resin ultrathin occlusal veneers had a significantly higher fatigue resistance when compared to the ceramic veneers.

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

removed because they were book chapters, and 352 studies were excluded as they did not meet the eligibility criteria. The remaining 19 studies were subjected to a full-text review. Three studies were excluded because they employed finite element analyses, 1 study was a systematic review, and 9 studies did not meet the inclusion criteria. Therefore, a total of 6 studies were included in the systematic review. Three of these examined the fracture resistance,[23,25,29] while the remaining 3 examined the fatigue resistance.[17,19,30]

Risk of bias

Of the 6 included studies, 2 were identified as having a medium risk of bias,[23,25] while 4 had a high risk of bias.[17,19,29,30] The results are described in Table 2, according to the parameters considered in the analysis.

The most commonly identified risks of bias among the studies were a lack of blinding of the testing machine operator, a lack of description of the sample size calculation, and tooth preparation performed by the same operator.

Main findings

The characteristics of the materials used in the studies included in this systematic review are presented in Table 3. The fracture resistance of CAD/CAM occlusal veneers was evaluated in 3 studies.[23,25,29] Two of these studies had restorations of the same thickness and used thermocycling.[25,29] The results indicate that there is no statistically significant difference between the use of CAD/CAM composite occlusal veneers and CAD/CAM lithium disilicate veneers (Table 4).

Table 2. Assessment of the risk of bias

Study	Teeth randomization	Teeth free of caries or restoration	Materials used according to the manufacturer's instructions	Teeth with similar dimensions	Tooth preparation performed by the same operator	Sample size calculation	Blinding of the operator of the testing machine	Risk of bias
Andrade et al. 2018[23]	Y	Y	Y	Y	N	N	N	medium
Al-Akhali et al. 2017[25]	N	Y	Y	Y	N	Y	N	medium
Al-Akhali et al. 2019[29]	Y	Y	Y	N	N	N	N	high
Heck et al. 2019[30]	N	Y	Y	Y	N	N	N	high
Magne et al. 2010[19]	N	Y	Y	N	N	N	N	high
Schlichting et al. 2011[17]	N	Y	Y	N	N	N	N	high

Y – yes; N – no.

Table 3. Characteristics of the materials used in the included studies

Material	Classification	Manufacturer	Composition
IPS e.max CAD	lithium disilicate glass ceramic	Ivoclar Vivadent AG, Schaan, Liechtenstein	SiO_2 (57.0–80.0%), Li_2O (11.0–19.0%), K_2O (0.0–13.0%), P_2O_5 (0.0–11.0%), ZrO_2 (0.0–8.0%), ZnO (0.0–8.0%), Al_2O_3 (0.0–5.0%), MgO (0.0–5.0%), coloring oxides (0.0–8.0%)
Lava Ultimate	resin nanoceramic	3M ESPE, St. Paul, USA	silica nanomers (20 nm), zirconia nanomers (4–11 nm), nanocluster particles derived from the nanomers (0.6–10 nm), silane coupling agent, resin matrix (Bis-GMA, Bis-EMA, UDMA, and TEGDMA)
VITA ENAMIC	hybrid ceramic (glass ceramic in a resin interpenetrating matrix)	VITA Zahnfabrik, Bad Säckingen, Germany	inorganic ceramic content: 86 wt% (silicon dioxide (58–63%), aluminum oxide (20–23%), sodium oxide (9–11%), potassium oxide (4–6%), boron trioxide (0.5–2%), zirconia (<1%), calcium oxide (<1%)) organic polymer content: 14 wt% (UDMA and TEGDMA)
Paradigm MZ100	zirconia–silica ceramic in a resin interpenetrating matrix	3M ESPE, St. Paul, USA	Paradigm MZ100: 85 wt% ultrafine zirconia–silica ceramic particles that reinforce a highly cross-linked polymer matrix polymer matrix: Bis-GMA and TEGDMA

Bis-GMA – bisphenol A-glycidyl methacrylate; Bis-EMA –bisphenol-A ethoxylated dimethacrylate; UDMA – urethane dimethacrylate; TEGDMA – triethylene glycol dimethacrylate.

On the other hand, the fatigue resistance was evaluated according to the survival rate in 3 investigations,[17,19,30] with 1 study demonstrating no statistically significant difference in the survival rate.[30] However, in 2 studies, the survival rate was higher in CAD/CAM composite resin occlusal restorations (Table 5).

The results of this study indicate that the use of CAD/CAM composite and lithium disilicate occlusal veneers in worn posterior teeth is a viable option. Due to the heterogeneity and risk of bias, a quantitative analysis could not be performed.

Discussion

This systematic review demonstrates that CAD/CAM composite resin occlusal veneers exhibit fracture resistance values ranging from 1,018.5 N to 3,584.0 N, even in thin veneers (0.5–1.5 mm), which exceed the maximum bite force of patients without parafunctional habits (424–630 N).[23] These results are consistent with those of a systematic review[31] that recommends the use of CAD/CAM composite resin occlusal veneers less than 1-mm thick and lithium disilicate veneers from 0.7 mm to 1.5 mm thick. In their study, Maeder et al.[32] evaluated various materials and found that VITA ENAMIC®, with a thickness of 0.5 mm, required a 800-N greater load than the maximum bite force to produce a crack in the veneer. Therefore, this material reaches high values of fracture resistance, which can be attributed to its composition, consisting of a hybrid structure with 2 interpenetrated ceramic and polymeric networks, and resulting in a Weibull modulus of 20. This is in relation to the fracture range, reliability and strength of the material.[33] Ioannidis et al.[34] also reported that 0.5-mm thick VITA ENAMIC occlusal veneers have load capacity values above the normal force intervals. Johnson et al.[16] compared CAD/CAM composite resin occlusal veneers, including Lava™ Ultimate and Paradigm™ MZ100 with varying thicknesses (0.3 mm, 0.6 mm and 1 mm).

Table 4. Fracture resistance observed in the included studies

Study	Variables					Results					
	material	restoration thickness [mm]	cyclic mechanical loading	thermocycling	antagonist material	fracture resistance [N] M±SD	fracture resistance without thermomechanical loading [N]		fracture resistance after thermomechanical loading [N]		conclusions
							M±SD	Me	M±SD	Me	
Andrade et al. 2018[23]	IPS e.max CAD	0.6	1,000,000 cycles at 1-Hz frequency in distilled water at 37°C, a load of 200 N	no thermocycling	a metal sphere with a 6-mm diameter	3,067 ±933	–	–	–	–	A significantly higher fracture resistance was obtained for 1.5-mm IPS e.max CAD than for the other experimental groups ($p = 0.027$). There was no significant difference between 0.6-mm VITA ENAMIC, Lava Ultimate and IPS e.max CAD ($p = 0.050$). The fracture resistance of the sound teeth (3,991 N) did not differ significantly from that of the experimental groups ($p = 0.199$).
	IPS e.max CAD	1.5				4,995 ±855	–	–	–	–	
	VITA ENAMIC	0.6				2,973 ±635	–	–	–	–	
	VITA ENAMIC	1.5				3,540 ±986	–	–	–	–	
	Lava Ultimate	0.6				3,384 ±922	–	–	–	–	
	Lava Ultimate	1.5				3,584 ±954	–	–	–	–	
Al-Akhali et al. 2017[25]	IPS e.max CAD	0.5	1,200,000 mechanical chewing cycles, a vertical load of 98 N	between 5°C and 55°C in distilled water, 30-s dwell time at each temperature with a total of 5,500 thermal cycles at a loading cycle frequency of 2.4 Hz	steatite ceramic balls with a 6-mm diameter	–	1,408.8 ±215.8	1,335.0	1,545.0 ±175.2	1,560.0	Thermodynamic loading significantly increased the fracture resistance of VITA ENAMIC ($p ≤ 0.031$). Without thermodynamic loading, lithium disilicate showed a significantly higher fracture resistance than VITA ENAMIC ($p ≤ 0.015$). After thermodynamic loading, no statistically significant difference was observed between the groups ($p ≤ 0.291$).
	VITA ENAMIC	0.5				–	1,018.5 ±155.5	1,005.0	1,321.0 ±269.1	1,310.0	
Al-Akhali et al. 2019[29]	IPS e.max CAD	0.5	1,200,000 mechanical chewing cycles, a vertical load of 98 N	between 5°C and 55°C in distilled water, 30-s dwell time at each temperature with a total of 5,500 thermal cycles at a loading cycle frequency of 2.4 Hz	steatite ceramic balls with a 6-mm diameter	–	806.1 ±186.9	782.5	470.8 ±428.2	328.5	Thermomechanical fatigue significantly reduced the fracture strength of VITA ENAMIC ($p = 0.047$). Lithium disilicate exhibited no significant reduction in the fracture strength after thermomechanical fatigue. There was no statistically significant difference between the groups with regard to thermomechanical fatigue.
	VITA ENAMIC	0.5				–	767.1 ±130.9	769.5	349.9 ±350.5	98.0	

M – mean; *Me* – median; *SD* – standard deviation.

Table 5. Fatigue resistance observed in the included studies based on the survival rate

Study	Material	Restoration thickness	Antagonist material	Cyclic mechanical loading	Survival rate [%]	Results
Heck et al. 2019[30]	IPS e.max CAD	0.3–0.5 mm	5-mm highly compacted oxide ceramic (Degussit balls; FRIALIT-DEGUSSIT, Mannheim, Germany)	1,000,000 masticatory cycles with a loading force of 50 N and 100 N and a frequency of 1 Hz	100	There was no significant difference between IPS e.max CAD and Lava Ultimate ($p = 0.317$) .
	Lava Ultimate				95	
Magne et al. 2010[19]	IPS e.max CAD	1.2 mm at the central groove	7-mm-diameter composite resin sphere (Z100™ MP; 3M ESPE, St. Paul, USA) postpolymerized at 100°C for 5 min	cyclic load applied at a frequency of 5 Hz and 1,400 N, at a maximum of 185,000 cycles	30	A higher fatigue resistance was observed in Paradigm MZ100 compared to IPS e.max CAD ($p = 0.002$).
	Paradigm MZ100				100	
Schlichting et al. 2011[17]	IPS e.max CAD	0.6 mm at the central groove	7-mm-diameter composite resin sphere (Z100™ MP; 3M ESPE) postpolymerized at 100°C for 5 min	cyclic load applied at a frequency of 5 Hz and 1,400 N, at a maximum of 185,000 cycles	0	A higher fatigue resistance was observed in Paradigm MZ100 compared to IPS e.max CAD ($p < 0.001$). XR experimental blocks were significantly stronger than IPS e.max CAD ($p < 0.001$), but not different from Paradigm MZ100 ($p = 0.030$).
	Paradigm MZ100				60	
	XR experimental blocks				100	

The obtained fracture resistance values were higher than normal masticatory forces, even at the minimum thickness of 0.3 mm. Therefore, minimum thickness, non-ceramic occlusal veneers could be considered a restorative option in patients with normal masticatory loads. However, in patients with parafunctional habits and excessive loads (780–1,120 N), complications may arise, including restoration dislodgment and fracture.[16,23]

In terms of fatigue resistance, there were no statistically significant differences between CAD/CAM lithium disilicate and composite resin occlusal veneers with a thickness of 0.3–0.5 mm, including IPS e.max CAD and Lava Ultimate, respectively. In the studies conducted by Magne et al.[19] and Schlichting et al.,[17] Paradigm MZ100 occlusal veneers with thicknesses of 1.2 mm and 0.6 mm demonstrated higher resistance values than IPS e.max CAD, applying a final load of 1,400 N in both studies. On the other hand, in the study by Schlichting et al.,[17] XR experimental blocks were also significantly stronger than IPS e.max CAD, but not different from MZ100. The results of these studies suggest that higher flexural strength does not necessarily correspond to higher load resistance.[19] According to the studies included in this systematic review, CAD/CAM composite resin occlusal veneers have a survival rate of 95–100%, despite their lower flexural strength than lithium disilicate veneers. For example, lithium disilicate has a flexural strength of 360–440 mPa, in contrast to MZ100, which has a flexural strength of 150 mPa.[17,19,35] Neither of these values is correlated with the respective survival rate. Similarly, Lava Ultimate and VITA ENAMIC blocks show flexural strength values of 200 mPa and 150–160 mPa, respectively.[23] The elastic moduli of Lava Ultimate (13 gPa) and VITA ENAMIC (30 gPa) are close to that of dentin (20.3 gPa), suggesting that they may influence the performance of restorations,[23] since the elasticity of dentin compensates for the stiffness of enamel, cushioning it against masticatory forces. Consequently, the distribution of stress within a restored tooth during mastication depends on this property.[36,37] However, it should be noted that thermocycling was not employed in the studies conducted by Magne et al.[19] and Schlichting et al.[17]

In vitro studies that use thermocycling are of great importance, as the procedure enables the simulation of the physiological conditions and temperature changes in the oral environment, which can result in physicochemical alterations in dental materials.[25,32,38] The study of Al-Akhali et al.[29] evaluated restoration survival by subjecting specimens to thermocycling for 1,200,000 cycles, which simulates 5 years of clinical service.[39,40] The results indicated low survival rates for both VITA ENAMIC and IPS e.max CAD blocks (37.5% and 50%, respectively). However, the authors of the study posit that the self-etch protocol reduced the fracture resistance of the CAD/CAM composite resin and lithium disilicate ceramic blocks. Therefore, enamel etching is required when placing occlusal veneers, since the self-etch technique results in an insufficient and unstable bond between the veneer and the tooth.[29] Self-etch adhesive systems produce a superficial enamel etching with reduced microporosity for resin infiltration, while orthophosphoric acid creates a porous enamel surface 5–50 μm deep. The poor etchability of self-etching adhesive systems on enamel can lead to pigmentation at the enamel margins, which may affect aesthetics, and could also be responsible for restoration dislodgement, marginal leakage and secondary caries, because self-etching does not achieve lasting adhesion to the enamel. Therefore, self-etching adhesive systems should be preceded by selective enamel etching with orthophosphoric acid.[41–45]

Occlusal veneers have been proposed as an alternative to full-coverage restorations for the treatment of worn posterior teeth, based on the results of several studies demonstrating their satisfactory mechanical properties.[17,21] Glass-ceramics, used in their manufacture, demonstrate several advantages, such as color stability, biocompatibility, durability, favorable translucency, chemical stability, reduction in the accumulation of bacterial plaque, and adequate marginal adjustment. However, they also have disadvantages, such as chipping, porosity and microstructural defects.[30,46–50] The CAD/CAM composite resin blocks are advantageous due to the low wear of the opposing teeth, a dentin-like elastic modulus, low cost, and the possibility of repair. Some disadvantages of this material include its tendency to absorb water, as well as its susceptibility to chemical and mechanical degradation.[23,51,52]

A comparative analysis of the fatigue and fracture resistance of CAD/CAM composite resin and CAD/CAM lithium disilicate blocks revealed that both materials, with a thickness ranging from 0.5 mm to 1.5 mm, are suitable for the treatment of occlusally worn teeth using an etch-and-rinse bonding procedure. However, these results should be interpreted with caution, as the present review revealed some limitations. The majority of the included studies showed a high risk of bias, as they did not clarify whether the extracted teeth were prepared by the same operator, and only 1 study mentioned the sample size calculation.[25] Additionally, variables such as the number of cycles, the load applied and veneer thickness were not consistent across all the included studies. It should also be noted that no clinical trials were identified during the search, as all included studies were conducted in vitro. Therefore, simulating the oral environment is challenging. Nevertheless, only 2 studies[25,29] used thermocycling, and it has been suggested that clinical studies be conducted with long-term follow-up. It is therefore recommended that future studies adopt a standardized methodology. Although the studies included in this review compared CAD/CAM lithium disilicate blocks (IPS e.max CAD; Ivoclar Vivadent AG, Schaan, Liechtenstein) with CAD/CAM composite resin blocks (Lava™ Ultimate, 3M ESPE, St. Paul, USA; VITA ENAMIC®, VITA Zahnfabrik, Bad Säckingen, Germany; Paradigm™ MZ100 Block, 3M ESPE), it should be noted that there are more CAD/CAM composite resin materials, such as Grandio blocs (VOCO GmbH, Cuxhaven, Germany), with high filler content (86% w/w), a high elastic (18 gPa) and flexural (290 mPa) modulus, a fracture resistance of 2,500 N, and a bite force that exceeds that of patients with parafunction.[53,54] Another notable material is BRILLIANT Crios (Coltène AG, Altstätten, Switzerland), which has an elastic modulus of 10 gPa and a fracture resistance of 1,255 N at a thickness of 1 mm.[50] However, to date, no studies have been conducted to compare occlusal veneers fabricated from these materials with lithium disilicate veneers. Due to the wide heterogeneity of the included studies, a meta-analysis could not be performed.

Conclusions

Computer-aided design/computer-aided manufacturing composite resin occlusal veneers exhibit similar mechanical performance in terms of fatigue and fracture resistance to CAD/CAM lithium disilicate veneers. Both types of veneers are suitable for use on worn posterior teeth. The CAD/CAM composite resin occlusal veneers are economical and repairable, while CAD/CAM lithium disilicate occlusal veneers have better color stability and reduced plaque accumulation. The optimal thickness for CAD/CAM composite resin and lithium disilicate occlusal veneers is 0.5–1.5 mm. Additionally, an etch-and-rinse or self-etch adhesive system with selective etching of the surface of the dental substrate should be used. It is recommended that randomized clinical studies be conducted on this topic.

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.

ORCID iDs

Karelys Del Cisne Maldonado https://orcid.org/0000-0003-4498-4032
Juan Andres Espinoza https://orcid.org/0000-0002-8574-1961
Daniela Andrea Astudillo https://orcid.org/0000-0002-8154-0492
Bolivar Andres Delgado https://orcid.org/0000-0001-5586-2829
Wilson Daniel Bravo https://orcid.org/0000-0002-9431-1808

References

1. Lambrechts P, Braem M, Vuylsteke-Wauters M, Vanherle G. Quantitative in vivo wear of human enamel. *J Dent Res.* 1989;68(12):1752–1754. doi:10.1177/00220345890680120601
2. Ray DS, Wiemann AH, Patel PB, Ding X, Kryscio RJ, Miller CS. Estimation of the rate of tooth wear in permanent incisors: A cross-sectional digital radiographic study. *J Oral Rehabil.* 2015;42(6):460–466. doi:10.1111/joor.12288
3. Loomans B, Opdam N, Attin T, et al. Severe tooth wear: European consensus statement on management guidelines. *J Adhes Dent.* 2017;19(2):111–119. doi:10.3290/j.jad.a38102
4. Salas MMS, Nascimento GG, Huysmans MC, Demarco FF. Estimated prevalence of erosive tooth wear in permanent teeth of children and adolescents: An epidemiological systematic review and meta-regression analysis. *J Dent.* 2015;43(1):42–50. doi:10.1016/j.jdent.2014.10.012
5. Bartlett D, Ganss C, Lussi A. Basic Erosive Wear Examination (BEWE): A new scoring system for scientific and clinical needs. *Clin Oral Investig.* 2008;12 Suppl 1(Suppl 1):S65–S68. doi:10.1007/s00784-007-0181-5
6. Wetselaar P, Lobbezoo F. The Tooth Wear Evaluation System: A modular clinical guideline for the diagnosis and management planning of worn dentitions. *J Oral Rehabil.* 2016;43(1):69–80. doi:10.1111/joor.12340

7. Edelhoff D, Beuer F, Schweiger J, Brix O, Stimmelmayr M, Guth JF. CAD/CAM-generated high-density polymer restorations for the pretreatment of complex cases: A case report. *Quintessence Int*. 2012;43(6):457–467. PMID:22532953.

8. Zero DT, Lussi A. Erosion – Chemical and biological factors of importance to the dental practitioner. *Int Dent J*. 2005;55(4 Suppl 1):285–290. doi:10.1111/j.1875-595X.2005.tb00066.x

9. Bartlett D, Phillips K, Smith B. A difference in perspective – the North American and European interpretations of tooth wear. *Int J Prosthodont*. 1999;12(5):401–408. PMID:10709520.

10. Bartlett DW. The role of erosion in tooth wear: Aetiology, prevention and management. *Int Dent J*. 2005;55(4 Suppl 1):277–284. doi:10.1111/j.1875-595X.2005.tb00065.x

11. West NX, Joiner A. Enamel mineral loss. *J Dent*. 2014;42 Suppl 1:S2–S11. doi:10.1016/S0300-5712(14)50002-4

12. Muts EJ, van Pelt H, Edelhoff D, Krejci I, Cune M. Tooth wear: A systematic review of treatment options. *J Prosthet Dent*. 2014;112(4):752–759. doi:10.1016/j.prosdent.2014.01.018

13. van den Breemer CR, Vinkenborg C, van Pelt H, Edelhoff D, Cune MS. The clinical performance of monolithic lithium disilicate posterior restorations after 5, 10, and 15 years: A retrospective case series. *Int J Prosthodont*. 2017;30(1):62–65. doi:10.11607/ijp.4997

14. Vailati F, Belser UC. Full-mouth adhesive rehabilitation of a severely eroded dentition: The three-step technique. Part 2. *Eur J Esthet Dent*. 2008;3(2):128–146. PMID:19655527.

15. Vaidyanathan TK, Vaidyanathan R. Recent advances in the theory and mechanism of adhesive resin bonding to dentin: A critical review. *J Biomed Mater Res B Appl Biomater*. 2009;88(2):558–578. doi:10.1002/jbm.b.31253

16. Johnson AC, Versluis A, Tantbirojn D, Ahuja S. Fracture strength of CAD/CAM composite and composite-ceramic occlusal veneers. *J Prosthodont Res*. 2014;58(2):107–114. doi:10.1016/j.jpor.2014.01.001

17. Schlichting LH, Maia HP, Baratieri LN, Magne P. Novel-design ultra-thin CAD/CAM composite resin and ceramic occlusal veneers for the treatment of severe dental erosion. *J Prosthet Dent*. 2011;105(4):217–226. doi:10.1016/S0022-3913(11)60035-8

18. Schlichting LH, Resende TH, Reis KR, Magne P. Simplified treatment of severe dental erosion with ultrathin CAD-CAM composite occlusal veneers and anterior bilaminar veneers. *J Prosthet Dent*. 2016;116(4):474–482. doi:10.1016/j.prosdent.2016.02.013

19. Magne P, Schlichting LH, Maia HP, Baratieri LN. In vitro fatigue resistance of CAD/CAM composite resin and ceramic posterior occlusal veneers. *J Prosthet Dent*. 2010;104(3):149–157. doi:10.1016/S0022-3913(10)60111-4

20. Magne P, Stanley K, Schlichting LH. Modeling of ultrathin occlusal veneers. *Dent Mater*. 2012;28(7):777–782. doi:10.1016/J.DENTAL.2012.04.002

21. Sasse M, Krummel A, Klosa K, Kern M. Influence of restoration thickness and dental bonding surface on the fracture resistance of full-coverage occlusal veneers made from lithium disilicate ceramic. *Dent Mater*. 2015;31(8):907–915. doi:10.1016/J.DENTAL.2015.04.017

22. Krummel A, Garling A, Sasse M, Kern M. Influence of bonding surface and bonding methods on the fracture resistance and survival rate of full-coverage occlusal veneers made from lithium disilicate ceramic after cyclic loading. *Dent Mater*. 2019;35(10):1351–1359. doi:10.1016/j.dental.2019.07.001

23. Andrade JP, Stona D, Bittencourt HR, Borges GA, Burnett Júnior LH, Spohr AM. Effect of different computer-aided design/computer-aided manufacturing (CAD/CAM) materials and thicknesses on the fracture resistance of occlusal veneers. *Oper Dent*. 2018;43(5):539–548. doi:10.2341/17-131-L

24. Ilie N, Hilton TJ, Heintze SD, et al. Academy of Dental Materials guidance – Resin composites: Part I – Mechanical properties. *Dent Mater*. 2017;33(8):880–894. doi:10.1016/j.dental.2017.04.013

25. Al-Akhali M, Chaar MS, Elsayed A, Samran A, Kern M. Fracture resistance of ceramic and polymer-based occlusal veneer restorations. *J Mech Behav Biomed Mater*. 2017;74:245–250. doi:10.1016/j.jmbbm.2017.06.013

26. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71

27. Faggion CM. Guidelines for reporting pre-clinical in vitro studies on dental materials. *J Evid Based Dent Pract*. 2012;12(4):182–189. doi:10.1016/j.jebdp.2012.10.001

28. Sarkis-Onofre R, Skupien JA, Cenci MS, Moraes RR, Pereira-Cenci T. The role of resin cement on bond strength of glass-fiber posts luted into root canals: A systematic review and meta-analysis of in vitro studies. *Oper Dent*. 2014;39(1):E31–E44. doi:10.2341/13-070-LIT

29. Al-Akhali M, Kern M, Elsayed A, Samran A, Chaar MS. Influence of thermomechanical fatigue on the fracture strength of CAD-CAM-fabricated occlusal veneers. *J Prosthet Dent*. 2019;121(5):644–650. doi:10.1016/j.prosdent.2018.07.019

30. Heck K, Paterno H, Lederer A, Litzenburger F, Hickel R, Kunzelmann KH. Fatigue resistance of ultrathin CAD/CAM ceramic and nanoceramic composite occlusal veneers. *Dent Mater*. 2019;35(10):1370–1377. doi:10.1016/j.dental.2019.07.006

31. Albelasy EH, Hamama HH, Tsoi JKH, Mahmoud SH. Fracture resistance of CAD/CAM occlusal veneers: A systematic review of laboratory studies. *J Mech Behav Biomed Mater*. 2020;110:103948. doi:10.1016/j.jmbbm.2020.103948

32. Maeder M, Pasic P, Ender A, Özcan M, Benic GI, Ioannidis A. Load-bearing capacities of ultra-thin occlusal veneers bonded to dentin. *J Mech Behav Biomed Mater*. 2019;95:165–171. doi:10.1016/j.jmbbm.2019.04.006

33. Dirxen C, Blunck U, Preissner S. Clinical performance of a new biomimetic double network material. *Open Dent J*. 2013;7:118–122. doi:10.2174/1874210620130904003

34. Ioannidis A, Mühlemann S, Özcan M, Hüsler J, Hämmerle CHF, Benic GI. Ultra-thin occlusal veneers bonded to enamel and made of ceramic or hybrid materials exhibit load-bearing capacities not different from conventional restorations. *J Mech Behav Biomed Mater*. 2019;90:433–440. doi:10.1016/j.jmbbm.2018.09.041

35. Ma L, Guess PC, Zhang Y. Load-bearing properties of minimal-invasive monolithic lithium disilicate and zirconia occlusal onlays: Finite element and theoretical analyses. *Dent Mater*. 2013;29(7):742–751. doi:10.1016/J.DENTAL.2013.04.004

36. Kinney JH, Balooch M, Marshall GW, Marshall SJ. A micromechanics model of the elastic properties of human dentine. *Arch Oral Biol*. 1999;44(10):813–822. doi:10.1016/S0003-9969(99)00080-1

37. Bowen RL, Rodriguez MS. Tensile strength and modulus of elasticity of tooth structure and several restorative materials. *J Am Dent Assoc*. 1962;64(3):378–387. doi:10.14219/jada.archive.1962.0090

38. Oyafuso DK, Özcan M, Bottino MA, Itinoche MK. Influence of thermal and mechanical cycling on the flexural strength of ceramics with titanium or gold alloy frameworks. *Dent Mater*. 2008;24(3):351–356. doi:10.1016/J.DENTAL.2007.06.008

39. Steiner M, Mitsias ME, Ludwig K, Kern M. In vitro evaluation of a mechanical testing chewing simulator. *Dent Mater*. 2009;25(4):494–499. doi:10.1016/j.dental.2008.09.010

40. Kern M, Strub JR, Lü XY. Wear of composite resin veneering materials in a dual-axis chewing simulator. *J Oral Rehabil*. 1999;26(5):372–378. doi:10.1046/J.1365-2842.1999.00416.X

41. Szesz A, Parreiras S, Reis A, Loguercio A. Selective enamel etching in cervical lesions for self-etch adhesives: A systematic review and meta-analysis. *J Dent*. 2016;53:1–11. doi:10.1016/j.jdent.2016.05.009

42. Bedran-Russo A, Leme-Kraus AA, Vidal CMP, Teixeira EC. An overview of dental adhesive systems and the dynamic tooth – Adhesive interface. *Dent Clin North Am*. 2017;61(4):713–731. doi:10.1016/j.cden.2017.06.001

43. van Meerbeek B, Yoshihara K, van Landuyt K, Yoshida Y, Peumans M. From Buonocore's pioneering acid-etch technique to self-adhering restoratives. A status perspective of rapidly advancing dental adhesive technology. *J Adhes Dent*. 2020;22(1):7–34. doi:10.3290/j.jad.a43994

44. Sheets JL, Wilcox CW, Barkmeier WW, Nunn ME. The effect of phosphoric acid pre-etching and thermocycling on self-etching adhesive enamel bonding. *J Prosthet Dent*. 2012;107(2):102–108. doi:10.1016/S0022-3913(12)60033-X

45. Erickson RL, Barkmeier WW, Kimmes NS. Bond strength of self-etch adhesives to pre-etched enamel. *Dent Mater*. 2009;25(10):1187–1194. doi:10.1016/J.DENTAL.2009.04.004

46. Gracis S, Thompson VP, Ferencz JL, Silva NRFA, Bonfante EA. A new classification system for all-ceramic and ceramic-like restorative materials. *Int J Prosthodont*. 2015;28(3):227–235. doi:10.11607/ijp.4244

47. Stappert CFJ, Abe P, Kurths V, Gerds T, Strub JR. Masticatory fatigue, fracture resistance, and marginal discrepancy of ceramic partial crowns with and without coverage of compromised cusps. *J Adhes Dent*. 2008;10(1):41–48. PMID:18389735.

48. Yu W, Guo K, Zhang B, Weng W. Fracture resistance of endodontically treated premolars restored with lithium disilicate CAD/CAM crowns or onlays and luted with two luting agents. *Dent Mater J*. 2014;33(3):349–354. doi:10.4012/dmj.2013-240

49. Rizzante FAP, Soares-Rusu IBL, Senna SS, et al. Flexural strength of minimum thickness ceramic veneers manufactured with different techniques. *Quintessence Int*. 2020;51(4):268–273. doi:10.3290/j.qi.a44147

50. Zimmermann M, Ender A, Egli G, Özcan M, Mehl A. Fracture load of CAD/CAM-fabricated and 3D-printed composite crowns as a function of material thickness. *Clin Oral Investig*. 2019;23(6):2777–2784. doi:10.1007/s00784-018-2717-2

51. Magne P, Carvalho AO, Bruzi G, Giannini M. Fatigue resistance of ultrathin CAD/CAM complete crowns with a simplified cementation process. *J Prosthet Dent*. 2015;114(4):574–576. doi:10.1016/j.prosdent.2015.04.014

52. Kunzelmann KH, Jelen B, Mehl A, Hickel R. Wear evaluation of MZ100 compared to ceramic CAD/CAM materials. *Int J Comput Dent*. 2001;4(3):171–184. PMID:11862884.

53. Rosentritt M, Preis V, Behr M, Hahnel S. Influence of preparation, fitting, and cementation on the vitro performance and fracture resistance of CAD/CAM crowns. *J Dent*. 2017;65:70–75. doi:10.1016/J.JDENT.2017.07.006

54. Rosentritt M, Krifka S, Strasser T, Preis V. Fracture force of CAD/CAM resin composite crowns after in vitro aging. *Clin Oral Investig*. 2020;24(7):2395–2401. doi:10.1007/s00784-019-03099-1

Review

Pain and root resorption due to surgical interventions to accelerate tooth movement in orthodontics: A systematic review and meta-analysis

Mariano Ortiz-Pizarro[1,A–F], Marcos Jimmy Carruitero-Honores[2,3,C–F], Silvio Augusto Bellini-Pereira[4,D–F], Aron Aliaga-Del Castillo[4,D–F]

[1] School of Medicine, César Vallejo University, Trujillo, Peru

[2] Faculty of Medicine, Antenor Orrego Private University, Trujillo, Peru

[3] School of Dentistry, Santo Toribio de Mogrovejo Catholic University, Chiclayo, Peru

[4] Department of Orthodontics, Baurú Dental School, University of São Paulo, 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

Abstract

Background. There are several publications that show the efficacy of surgical interventions in accelerating the rate of tooth movement in orthodontics. Consequently, possible adverse effects must also be evaluated.

Objectives. The aim of the present study was to compare the perception of pain and root resorption between orthodontic treatment with a surgical acceleration intervention vs. conventional orthodontic treatment.

Material and methods. An electronic search was conducted in the MEDLINE, Scopus, Web of Science (WoS), ScienceDirect, Cochrane Library, and Virtual Health Library (VHL) databases up to September 12, 2022. Randomized or non-randomized, controlled, parallel-arm or split-mouth clinical trials were included. Fixed-and random-effects meta-analyses were performed with regard to heterogeneity. The risk of bias (RoB) was assessed using the RoB 2.0 and ROBINS-I tools.

Results. A total of 1,395 articles were initially retrieved, 40 studies were finally included in the review and 15 studies were eligible for quantitative analysis. The meta-analysis showed a significant difference in pain perception between acceleration surgery vs. conventional orthodontics at 24 h ($p = 0.040$); however, this difference was not significant at 7 days ($p = 0.080$). Overall, the patients who underwent any acceleration procedure presented significantly less resorption as compared to those who were applied conventional treatment ($p < 0.001$). A similar significant difference was found in retraction movements ($p < 0.001$) and alignment movements ($p = 0.030$).

Conclusions. In the first 24 h, surgical interventions for the acceleration of tooth movement produce a greater perception of pain as compared to conventional orthodontic treatment, but the perception is similar after 7 days. Acceleration surgery results in less root resorption – in alignment movements, and especially in retraction movements.

Keywords: pain, acceleration, orthodontic tooth movement, root resorption, oral surgical procedures

Introduction

In recent years, techniques for accelerating tooth movement in orthodontics have been demonstrated, and they have become an interesting option for adult patients who require fixed orthodontic treatment, but within a shorter period.[1–4] In general, acceleration interventions initiate a regional inflammatory process with temporary osteopenia due to increased osteoclastic activity, enabling the reduction of bone resistance with respect to tooth movement.[2,3] Surgical interventions may include techniques such as corticotomy – with or without laser, piezocision, discision, corticision, piezopuncture, and micro-osteoperforation.[4,5]

Ideally, this approach should allow clinicians to control both the level and location of inflammation, preventing negative side effects as much as possible.[5] However, it has also been established that the inflammatory mechanisms necessary to generate tooth movement share some characteristics with inflammatory processes that are not favorable for tissue integrity.[6–8]

Recently, a significant number of publications have reported evidence of the effect of surgical acceleration interventions in orthodontic treatment, showing favorable results with respect to the amount and rate of movement.[8–13] There are fewer and fewer clinical trials that evaluate, under a certain methodology, the adverse effects due to the inflammatory mechanisms of an acceleration intervention.[8–10] The perception of pain and root resorption are 2 important outcomes in terms of patient acceptance and long-term success of the intervention,[4,9] but there are very few systematic reviews that quantitatively report on these unfavorable outcomes,[9,13] making more studies necessary to be able to reach a consensus on the safety of acceleration interventions.

Therefore, the purpose of this systematic review was to summarize and analyze the available evidence regarding the effect of surgical interventions to accelerate tooth movement with respect to pain perception and root resorption as compared to conventional treatment.

Material and methods

This review followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines.[14] The focused question was: "Do surgical interventions to accelerate tooth movement produce a similar perception of pain and root resorption as compared to conventional orthodontic treatment?"

The inclusion criteria were established according to the PICO strategy. The population comprised adult and adolescent patients undergoing orthodontic treatment (P). Surgical techniques for tooth movement acceleration were considered as interventions (I). Conventional orthodontic treatment was considered the comparison (C). The outcomes were pain perception and root resorption (O).

Inclusion criteria

The inclusion criteria were as follows:
– clinical trials comparing a surgical intervention to accelerate tooth movement with conventional orthodontic treatment;
– clinical trials with the following outcomes evaluated – perception of pain or root resorption;
– randomized or non-randomized, controlled, parallel-arm or split-mouth clinical trials; and
– clinical trials in any language and without restrictions regarding the publication time.

Exclusion criteria

The exclusion criteria were as follows:
– clinical trials using more than one surgical acceleration intervention, an additional surgical technique or bone grafting in the experimental group;
– clinical trials using some surgical procedure in the comparison group;
– clinical trials with acceleration surgeries provided together with orthopedic or functional treatment; and
– observational studies, animal studies, case reports, books, editorials, and expert opinions.

Search strategy

An electronic literature search was carried out by 2 independent reviewers (M.O.P and M.J.C.H.), using the following databases: MEDLINE (via PubMed), Scopus, Web of Science (WoS), ScienceDirect, Cochrane Library, and Virtual Health Library (VHL). Handsearching was performed in other sources, such as Google Scholar to identify unpublished articles, orthodontic journals with an impact factor greater than 1, and through the reference list of each retrieved article. This review also shows the results of a supplemental search of gray literature through OpenGray and MedRxiv. The general search expression was as follows: (rapid* OR accelerat* OR speed*) AND ("tooth movement" OR orthod*) AND ("root resorption" OR "orthodontic resorption" OR "pain" OR "visual analog scale"). The search strategy used was modified according to the search syntax in each database. The literature search was performed without time restrictions, and the last date of the search was September 12, 2022.

Data collection

After the removal of duplicates, 2 independent reviewers (M.O.P and M.J.C.H.) selected the remaining articles in 2 phases. In the 1st phase, both reviewers examined the studies by title and abstract to determine retrieved articles that met the inclusion criteria. In case of disagreement, a decision was made by consensus through the participation of a third evaluator (S.A.B.P.), and then the articles

were incorporated. In the 2nd phase, the same reviewers performed a full-text evaluation of the pre-selected articles to determine their eligibility and proceed to data extraction.

Data extraction

Two independent reviewers (M.O.P and M.J.C.H.) extracted the information from the included articles using a standardized Microsoft Excel spreadsheet. The following data was extracted: title; first author; year of publication; study design; sample size; dental groups; gender and age of the participants; characteristics of the malocclusion; mechanics of movement and the applied force; type and details of the intervention; and characteristics of the evaluated outcomes (definition, measurement instrument, unit of measurement, and follow-up time). Any discrepancies or disagreement were resolved through the participation of a third investigator (S.A.B.P.).

Risk of bias

The risk of bias (RoB) assessment of the included studies was carried out using different tools depending on the study design. For randomized clinical trials (RCTs), the RoB 2.0 tool[15] of the Cochrane Collaboration was used, allowing the studies to be classified as being of low RoB, some concerns or high RoB. The ROBINS-I tool[16] was used to evaluate non-randomized studies (NRSs), allowing the studies to be classified into low, moderate, serious, critical RoB, or no information categories. Again, the RoB assessment was performed independently by 2 reviewers (M.O.P and M.J.C.H.), and any disagreement was resolved through discussion with a third author (S.A.B.P.).

Statistical analysis

The primary outcome was pain perception and the secondary outcome was root resorption. Quantitative data from studies with similar measurement methodologies and follow-up time for outcomes were pooled. For the perception of pain, a measurement interval of 24 h was considered for scales from 1 to 10, and 7 days for scales from 1 to 100, while the evaluation of root resorption was considered in linear millimeters, with a minimum follow-up period of 3 months, and according to tooth movements of alignment or retraction.

A meta-analysis was performed using a computer program (RevMan, v. 5.4), and the extracted data was expressed as continuous variables. The mean and standard deviation ($M \pm SD$) with a 95% confidence interval (CI) were used to estimate the treatment effect. Statistical significance for the hypothesis test was established at $p < 0.05$. A random-effects model was considered, while heterogeneity between the studies was estimated based on the χ^2, τ and I^2 statistics.

Results

The electronic search of the databases identified 1,310 articles published up to September 12, 2022. According to the established protocol, additional 85 articles were manually identified from other sources. Duplicate records were eliminated, and the remaining 997 studies were screened by title and abstract, with 834 records being excluded and 163 full-text articles reviewed for eligibility. Finally, after applying the exclusion criteria, 40 studies were included in the qualitative synthesis of the systematic review and 15 studies in the quantitative synthesis (Fig. 1).

Identification of studies via databases and registers

Identification

Records identified from:
- MEDLINE (n = 386)
- Scopus (n = 239)
- Web of Science (n = 33)
- ScienceDirect (n = 192)
- Cochrane Library (n = 3)
- Virtual Health Library (n = 457)
- other sources (n = 85)

→ Records removed before screening:
- duplicate records removed (n = 398)

Screening

Records screened (n = 997) → Records excluded (n = 834)

Reports sought for retrieval (n = 163) → Reports not retrieved (n = 0)

Reports assessed for eligibility (n = 163) → Reports excluded:
- different objectives (n = 79)
- different type or study design (n = 25)
- more than one intervention together (n = 11)
- intervention in orthopedic or functional treatment (n = 3)
- surgery in the control group (n = 5)

Included

Studies included in the qualitative synthesis (n = 40)
Studies included in the quantitative synthesis (n = 15)

Fig. 1. Flowchart of the study according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines

Study characteristics

Table 1 summarizes the main features of all included studies that evaluated treatment assisted by acceleration surgery vs. conventional orthodontic treatment.[1,2,17–54] Of the 40 included studies, 33 studies were RCTs,[18–20,22,25–34,36–54] of which 15 had a parallel-arm design[18,28–30,34,36,41,44,45,47,48,50,51,53,54] and 18 used a split-mouth design.[19,20,22,25–27,31–33,37–40,42,43,46,49,52] Likewise, 7 studies were NRSs,[1,2,17,21,23,24,35] of which 2 had a parallel-arm design[1,17] and 5 used a split-mouth design.[2,21,23,24,35]

Table 1. Characteristics of the included studies

Author, year	Study design	Groups (n)	Gender	Age [years]	Malocclusion/ treatment plan	Acceleration intervention	Mechanics of movement	Evaluation/ follow-up by outcome	Outcomes (statistical significance) pain	Outcomes (statistical significance) root resorption
Yavuz et al.[1] 2018	NRS parallel-arm	GPZO: 9, GDC:12, GC:14	35F	GPZO: 13–18, GDC: 13–18, GC: 13–19	Class I with crowding/without extractions	PZO DC	upper and lower alignment, self-ligating brackets	pain: VAS NR/30 days; resorption: panoramic X-ray/complete alignment	no quantitative comparison data	the difference between GPZO/GDC and GC was NS
Omidkhoda et al.[2] 2020	NRS split-mouth	GPZp: 17, GC:17	7M/10F	18.23 ±1.35	Class I and II-1/ bilateral extraction of maxillary 1PM	PZp	canine retraction, 150 grf, non-self-ligating brackets	pain: VAS 0–10/2 months; resorption: –	the difference between GPZp and GC was NS	none
Shoreibah et al.[17] 2012	NRS parallel-arm	GCT: 10, GC: 10	4M/16F	18.4–25.6	Class I, with a discrepancy of 3–5 mm/ without extractions	CT	lower anterior alignment	pain: –; resorption: periapical X-ray (mm)/6 months post-treatment	none	the difference between GCT and GC was NS
Alikhani et al.[18] 2013	RCT parallel-arm	GMOP: 10, GC:10	GMOP: 5M/5F, GC: 3M/7F	GMOP: 26.8, GC: 24.7	Class II-1/ extraction of maxillary 1PM	MOP	canine retraction, 100 grf	pain: rating scale 0–10/28 days; resorption: –	the difference between GMOP and GC was NS	none
Al-Naoum et al.[19] 2014	RCT split-mouth	GCT:30, GC: 30	15M/15M	20.04 ±3.63	Class II-1 and II-2/ extraction of maxillary 1PM	CT	canine retraction, 120 grf, non-self-ligating brackets	pain: Likert scale 1–4/7 days; resorption: –	no quantitative comparison data	none
Abbas et al.[20] 2016	RCT split-mouth	GPZO:10, GCT:10, GC:20	NR	15–25	Class II-1/ bilateral extraction of maxillary 1PM	PZO CT	canine retraction, 150 grf, non-self-ligating brackets	pain: –; resorption: CBCT (mm)/3 months	none	GC > GPZO and GCT
Patterson et al.[21] 2017	NRS split-mouth	GPZO:14, GC:14	6M/8F	16.17	NR/ bilateral extraction of maxillary 1PM	PZO	tipping forces in 1PM with 150 grf, self-ligating brackets	pain: –; resorption: CBCT (mm³)/28 days	none	GPZO > GC
Chan et al.[23] 2018	NRS split-mouth	GMOP: 20, GC: 20	8M/12F	15.4	malocclusion with crowding on each side of the maxilla/ extraction of 1PM	MOP	tipping forces in 1PM with 150 grf	pain: –; resorption: CBCT (mm³)/28 days	none	GMOP showed 48% higher resorption than GC
Elkalza et al.[24] 2018	NRS split-mouth	GMOP:8, GPZO: 8, GC: 16	NR	16–25	NR/ extraction of maxillary 1PM	MOP PZO	canine retraction, 150 grf, self-ligating brackets	pain: –; resorption: CBCT (mm³)/ complete retraction	none	the difference between GMOP and GC was NS; GPZO > GC
Sivarajan et al.[25] 2019	RCT split-mouth	GMOP: 30, GC: 30	7M/23F	22.2 ±3.72	NR/ extraction of the first 4 PMs	MOP	canine retraction 140–200 grf, non-self-ligating brackets	pain: Likert scale 1–5/16 weeks; resorption: –	GMOP > GC	none
Aboalnaga et al.[26] 2019	RCT split-mouth	GMOP: 18, GC: 18	0M/18F	20.50 ±3.85	NR/ bilateral extraction of maxillary 1PM	MOP	canine retraction, 150 grf, non-self-ligating brackets	pain: rating scale 1–10/7 days; resorption: CBCT (mm³) – Malmgren index 0–2/4 months	69% responded GMOP > GC	the difference between GMOP and GC was NS
Alqadasi et al.[27] 2019	RCT split-mouth	GMOP:8, GC: 8	NR	15–40	Class II-1/ extraction of maxillary 1PM	MOP	canine retraction, 150 grf, self-ligating brackets	pain: rating scale 0–10/28 days; resorption: CBCT (mm)/3 months	GMOP and GC had equal percentages of pain	the difference between GMOP and GC was NS
Bansal et al.[28] 2019	RCT parallel-arm	GMOP: 15, GC: 15	GMOP: 7M/8F, GC: 7M/8F	GMOP: 15.87 ±1.72, GC: 15.33 ±1.17	malocclusion with the irregularity index of 4–6 mm/ without extractions	MOP	antero-inferior alignment, non-self-ligating brackets	pain: VAS 0–100/7 days; resorption: CBCT (mm³)/6 months	GMOP > GC at T0	the difference between GMOP and GC was NS

Author, year	Study design	Groups (n)	Gender	Age [years]	Malocclusion/ treatment plan	Acceleration intervention	Mechanics of movement	Evaluation/ follow-up by outcome	Outcomes (statistical significance) pain	Outcomes (statistical significance) root resorption
Charavet et al.[29] 2019	RCT parallel-arm	GPZO: 12 GC: 12	GPZO: 5M/7F GC: 4M/8F	GPZO: 34 ±8 GC: 27 ±7	malocclusion with mild or moderate bimaxillary crowding without extractions	PZO	upper and lower alignment, self-ligating brackets	pain: VAS 0–10/7 days resorption: CBCT (mm)/ complete alignment	GPZO > GC	the difference between GPZO and GC was NS
Gibreal et al.[30] 2019	RCT parallel-arm	GPZO: 16 GC: 16	GPZO: 6M/10F GC: 7M/9F	GPZO: 20.86 ±1.90 GC: 21.27 ±1.87	Class II-1 with anterior inferior crowding/ extraction of maxillary 1PM	PZO	antero-inferior incisor alignment, self-ligating brackets	pain: VAS 0–100/28 days resorption: –	the difference between GPZO and GC was NS	none
Alfawal et al.[31] 2020	RCT split-mouth	GPZO: 16 GCTLz 16 GC: 16	GPZO: 7M/9F GCTLz 6M/10F	GPZO: 18.06 ±2.79 GCTLz 18.44 ±3.38	Class II-1/ bilateral extraction of maxillary 1PM	PZO CTLz	canine retraction, 150 grf, non-self-ligating brackets	pain: rating scale 0–10/7 days resorption: –	GPZO and GCTLz > GC at T1	none
Babanouri et al.[32] 2020	RCT split-mouth	GMOP: 25 GC: 25	11M/14F	15–45	Class I and II-1 with biprotrusion/ extraction of maxillary 1PM	MOP	canine retraction, 150 grf, non-self-ligating brackets	pain: VAS 0–10/2 days resorption: –	the difference between GMOP and GC was NS	none
Gulduren et al.[33] 2020	RCT split-mouth	GMOP: 9 GC: 9	5M/4F	GMOP: 21.8 GC: 17.7	Class I–II/ without extractions	MOP	superior molar distalization, 500 grf/frictionless	pain: VAS 0–10/20 days resorption: –	the difference between GMOP and GC was NS	none
Hatrom et al.[34] 2020	RCT parallel-arm	GPZO: 12 GC: 11	GPZO: 6M/6F GC: 5M/6F	GPZO: 19.8 ±3.1 GC: 20.4 ±4.1	Class II-1/ bilateral extraction of maxillary 1PM	PZO	en-mass retraction, 250 grf, non-self-ligating brackets	pain: rating scale 0–10/2 days resorption: CBCT (mm)/4 months	GPZO > GC at T0	GPZO < GC in central incisors and right canine
Ibrahim et al.[35] 2020	NRS split-mouth	GPZO: 10 GC: 10	NR	15–19	Class I and II-1/ bilateral extraction of maxillary 1PM	PZO	canine retraction, 150 grf, non-self-ligating brackets	pain: – resorption: CBCT (mm)/complete retraction	none	the difference between GPZO and GC was NS
Kundi et al.[36] 2020	RCT parallel-arm	GMOP: 15 GC: 15	14M/16F	GMOP: 27.5 ±4.4 GC: 28.4 ±4.5	Class II-1/ bilateral extraction of maxillary 1PM	MOP	canine retraction, 100 grf, non-self-ligating brackets	pain: rating scale 0–10/7 days resorption: –	GMOP > GC at T1 and T2	none
Mahmoudzadeh et al.[37] 2020	RCT split-mouth	GCTLz 12 GC: 12	3M/9F	18.91 ±3.87	NR/ bilateral extraction of maxillary 1PM	CTLz	canine retraction, 150 grf, non-self-ligating brackets	pain: VAS 0–10/7 days resorption: –	the difference between GCTLz and GC was NS	none
Raj et al.[38] 2020	RCT split-mouth	GPZO: 20 GC: 20	6M/14F	23.18 ±1.41	Class II-1/ bilateral extraction of maxillary 1PM	PZO	canine retraction, 150 grf, non-self-ligating brackets	pain: – resorption: CBCT (mm)/6 months	none	the difference between GPZO and GC was NS
Alqadasi et al.[39] 2021	RCT split-mouth	GMOP: 10 GPZO: 11 GC: 21	GMOP: 4M/6F GPZO: 5M/6F	20.89 ± 4.46	Class II-1/ bilateral extraction of maxillary PMs	MOP PZO	canine retraction, 150 grf, non-self-ligating brackets	pain: – resorption: CBCT (mm)/3 months	none	the difference between GMOP/ GPZO and GC was NS
Jaber et al.[40] 2021	RCT split-mouth	GCTLz 18 GC: 18	7M/11F	16.9 ±2.5	Class II-1/ bilateral extraction of maxillary 1PM	CTLz	canine retraction, 150 grf, non-self-ligating brackets	pain: Likert scale 1–4/7 days resorption: –	the difference between GCTLz and GC was NS	none
Ozkan and Arici41 2021	RCT parallel-arm	GMOP: 12 GC: 12	GMOP: 6M/6F GC: 6M/6F	GMOP: 17.27 ±1.20 GC: 18.13 ±1.20	Class I and II-1/ bilateral extraction of maxillary 1PM	MOP	canine retraction, 150 grf, non-self-ligating brackets	pain: VAS 0–100/1 day resorption: –	increased in both groups/ no quantitative comparison data	none
Ravi et al.[42] 2021	RCT split-mouth	GPZO: 15 GC: 15	NR	18–26	NR/ bilateral extraction of maxillary 1PM	PZO	canine retraction, 150 grf, non-self-ligating brackets	pain: – resorption: CBCT (mm)/3 months	none	the difference between GPZO and GC was NS
Raza et al.[43] 2021	RCT split-mouth	GCT: 10 GC: 10	mostly women, number NR	15–25	NR/ bilateral extraction of maxillary 1PM	CT	canine retraction, 150 grf, non-self-ligating brackets	pain: VAS 0–100/24 h and 7 days resorption: CBCT (mm)/complete retraction	24 h: GCT > GC 7 days: NS	GC > GCT

Author, year	Study design	Groups (n)	Gender	Age [years]	Malocclusion/ treatment plan	Acceleration intervention	Mechanics of movement	Evaluation/ follow-up by outcome	Outcomes (statistical significance) pain	Outcomes (statistical significance) root resorption
Shahrin et al.[44] 2021	RCT parallel-arm	GMOP: 14 GC: 14	5M/25F	22.66 ± 3.27	crowding with adiscrepancy of 5–8 mm/ extraction of 1PM	MOP	alignment and leveling of maxillary incisors	pain:– resorption: periapical X-ray (mm)/6 months	none	the difference between GMOP and GC was NS
Sirri et al.[45] 2021	RCT parallel-arm	GCTC: 26 GC: 26	14M/38F	GCTC: 21.30 ±1.49 GC: 21.46 ±1.76	crowding with the irregularity index of 2–6 mm/ without extractions	CTC	lower anterior alignment and leveling	pain:– resorption: CBCT (mm)/ complete alignment and leveling	none	the difference between GCTC and GC was NS
Thomas et al.[46] 2021	RCT split-mouth	GMOP: 33 GC: 33	9M/24F	22.10 ±2.19	Class I and II-1/ bilateral extraction of maxillary 1PM	MOP	canine retraction, 150 grf, non-self-ligating brackets	pain:– resorption: CBCT (mm)/3 months	none	the difference between GMOP and GC was NS
Alkasaby et al.[47] 2022	RCT parallel-arm	GMOP: 10 GC: 10	20F	GMOP: 18.1 ±1.2 GC: 18.0 ±1.1	space deficiency in the upper arch 5–8 mm/ without extractions	MOP	distalization of first molars by Fast Back/ 300 grf, frictionless	pain:– resorption: CBCT (mm)/ complete distalization	none	GMOP > GC (mesiobuccal roots)/ GC > GMOP (disto-buccal roots)
Arana et al.[48] 2022	RCT parallel-arm	GPZO: 7 GC: 8	mostly men, number NR	GPZO: 21.29 ±4.50 GC: 24.00 ±6.07	Class I, II or III with moderate irregularity/ without extractions	PZO	lower anterior alignment and leveling, self-ligating brackets	pain:– resorption: CBCT (mm)/ complete alignment and leveling	none	the difference between GPZO and GC was NS
Hawkins et al.[49] 2022	RCT split-mouth	GPZO: 20 GC: 20	8M/12F	18.70 ±1.12	crowding requiring the extraction of maxillary first PMs	PZO	canine retraction, 150 grf, self-ligating brackets	pain: VAS 0–100/0, 7 and 14 days resorption:–	GPZO had more pain but it was decreasing/ no comparison data	none
Al-Ibrahim et al.[50] 2022	RCT parallel-arm	GPZO: 22 GC: 22	GPZO: 4M/18F GC: 5M/17F	GPZO: 19.17 ±2.59 GC: 20.48 ±2.84	Class I with severe crowding(>6 mm)/ with extraction of maxillary first PMs	PZO	upper anterior alignment and leveling, self-ligating brackets	pain: VAS 0–100/1, 3 and 7 days resorption:–	1,3 and 7 days: GPZO > GC	none
Kumar et al.[51] 2024	RCT parallel-arm	GMOP: 10 GC: 10	GMOP: 4M/6F GC: 3M/7F	GMOP: 19.50 ±2.67 GC: 20.30 ±2.23	Class I protrusion or crowding/ with extraction of first PMs	MOP	retraction of maxillary and mandibular anterior teeth	pain: VAS 0–10/1, 7 and 14 days resorption:–	GMOP: a significant gradual decrease/ no comparison data	none
Li et al.[52] 2022	RCT split-mouth	GMOP: 20 GC: 20	NR	NR	NR/ bilateral extraction of maxillary 1PM	MOP	canine retraction, 150 grf, non-self-ligating brackets	pain: VAS 0–10 resorption:–	GMOP: a gradual decrease/ no comparison data	none
Sirri et al.[53] 2022	RCT parallel-arm	GCTC: 26 GC: 26	14M/38F	21.38 ± 1.05	crowding with the irregularity index of 2–6 mm/ without extractions	CTC	lower anterior alignment and leveling	pain: VAS 0–100/1, 7 and 14 days resorption:–	the difference between GCTC and GC was NS	none
Sultana et al.[54] 2022	RCT parallel-arm	GPZO: 6 GC: 7	GPZO: 0M/6F GC: 1M/6F	GPZO: 20.83 ±2.32 GC: 21.14 ±2.97	severe crowding requiring the extraction of maxillary first PMs	PZO	alignment and leveling of maxillary incisors	pain: VAS 0–10/0 and 7 days resorption:–	GPZO: post-operative pain was mild/ no comparison data	none

RCT – randomized clinical trial; NRS – non-randomized study; G – group; C – control; MOP – micro-osteoperforation; PZO – piezopuncture; M – male; F – female; PM – premolar; VAS – visual analog scale; CBCT – cone-beam computed tomography; T – follow-up time; >/< – significantly higher/lower; NS – statistically non-significant; NR – not reported.

Within the surgical acceleration techniques, 18 studies used micro-osteoperforation in 299 patients,[18,22–28,32,33,36,39,41,44,46,47,51,52] 16 studies used piezocision in 208 patients,[1,20,21,24,29–31,34,35,38,39,42,48–50,54] 3 studies used laser corticotomy in 46 patients,[31,37,40] 3 studies used traditional corticotomy in 50 patients,[17,19,43] 2 studies used corticision in 52 patients,[45,53] 1 study used discision in 12 patients,[1] and another study performed piezopuncture in 17 patients.[2]

Overall, across all the included studies, 26 studies assessed pain perception,[1,2,18,19,22,25–34,36,37,40,41,43,49–54] and 22 studies assessed root resorption.[1,17,20–24,26–29,34,35,38,39,42–48]

Risk of bias within the studies

Regarding RCTs, 16 studies were classified as low risk,[22,25,26,28,30–32,36,37,39,40,44–47,53] 6 were evaluated with some concerns[33,34,41,43,50,52] and 11 studies were classified as high risk of bias[18–20,27,29,38,42,48,49,51,54] (Fig. 2). The assessment of bias for NRSs is shown in Table 2, where 2 studies were classified as moderate risk[2,24], 3 serious[1,17,35] and 2 critical.[21,23]

Meta-analysis

Perception of pain

Two meta-analyses were performed regarding the units of measurement and the follow-up periods used for the primary outcome, pain perception. The 1st meta-analysis included 83 patients in 4 studies that used micro-osteoperforation along with an evaluation scale of 1–10. The analysis showed a statistically significant increase in the pain score of patients with acceleration surgeries as compared with those who underwent conventional treatment within a 24-hour observation period. The mean increase on the pain analog scale was 0.46 (95% CI: 0.02, 0.91; $p = 0.04$), and the studies showed homogeneity: $\chi^2 = 0.17$; df = 3 ($p = 0.98$); $I^2 = 0\%$ (Fig. 3A). Four studies that used a scale of 1–100 were included in the 2nd meta-analysis, with a total of 136 patients who underwent corticotomy, piezocision and corticision. Acceleration surgeries and conventional orthodontics produced similar pain scores over a 7-day observation period. The non-significant difference was 12.41 (95% CI: −1.32, 26.13; $p = 0.08$), and the studies showed heterogeneity: $\tau^2 = 187.32$; $\chi^2 = 88.42$; df = 3 ($p < 0.00001$); $I^2 = 97\%$ (Fig. 3B).

Root resorption

Three meta-analyses were performed to assess root resorption as a secondary outcome. In the 1st overall assessment, we included 9 studies using corticotomy, piezocision, corticision, or micro-osteoperforation in 235 patients. There was a significant decrease in resorption in patients who received any acceleration surgery as compared to those who underwent conventional treatment.
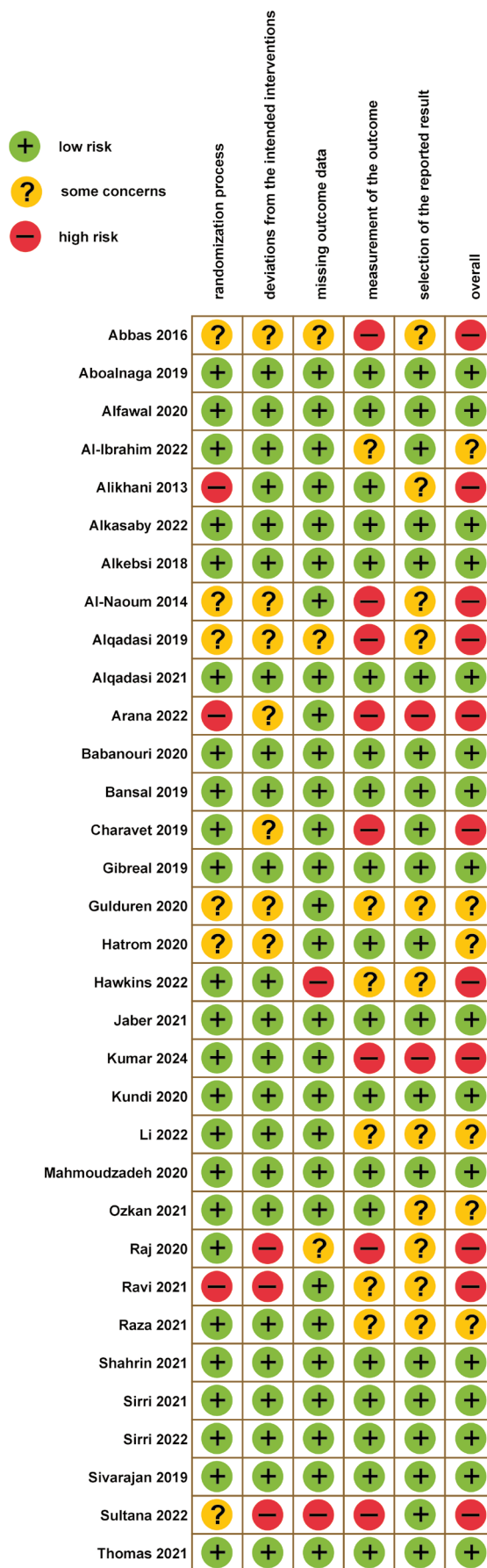

Fig. 2. Summary of the risk of bias (RoB) assessment for randomized controlled trials (RCTs) according to the RoB 2.0 tool

Table 2. Risk of bias (RoB) assessment for non-randomized studies (NRSs) according to the ROBINS-I tool

Study	Bias due to confounding	Bias in the selection of participants for the study	Bias in the classification of interventions	Bias due to deviations from the intended interventions	Bias due to missing data	Bias due to the measurement of the outcome	Bias due to the selection of the reported result	Overall risk of bias
Yavuz et al.[1] 2018	moderate	moderate	moderate	serious	no information	serious	serious	serious
Omidkhoda et al.[2] 2020	low	low	low	moderate	low	low	low	moderate
Shoreibah et al.[17] 2012	serious	moderate	serious	serious	no information	serious	moderate	serious
Patterson et al.[21] 2017	serious	low	serious	serious	low	critical	low	critical
Chan et al.[23] 2018	serious	low	serious	serious	low	critical	low	critical
Elkalza et al.[24] 2018	low	low	low	moderate	low	moderate	low	moderate
Ibrahim et al.[35] 2020	low	moderate	low	serious	no information	serious	low	serious

The mean decrease in root resorption was 0.24 mm (95% CI: −0.30, −0.17; $p < 0.00001$), and the studies showed homogeneity: $\chi^2 = 7.92$; df = 10 ($p = 0.64$); $I^2 = 0\%$ (Fig. 4A). Seven studies registered 155 patients and evaluated root resorption in retraction movements, showing a statistically significant decrease for patients who received corticotomy, piezocision or micro-osteoperforation as compared to conventional treatment. The mean decrease in root resorption was 0.26 mm (95% CI: −0.33, −0.18; $p < 0.00001$), and the included studies showed homogeneity: $\chi^2 = 5.68$; df = 8 ($p = 0.68$); $I^2 = 0\%$ (Fig. 4B). Finally, 2 studies evaluated root resorption in 80 patients during alignment movements. It was found that the patients who received corticision or micro-osteoperforation presented

a significant decrease in root resorption of 0.16 mm as compared to conventional treatment (95% CI: −0.30, −0.01; $p = 0.03$), and the included studies showed homogeneity: $\chi^2 = 0.83$; df = 1 ($p = 0.36$); $I^2 = 0\%$ (Fig. 4C).

Discussion

The present systematic review summarizes the evidence from randomized and non-randomized clinical trials that compared surgical interventions to accelerate tooth movement vs. conventional treatment without acceleration with respect to adverse effects, such as pain perception and root resorption.
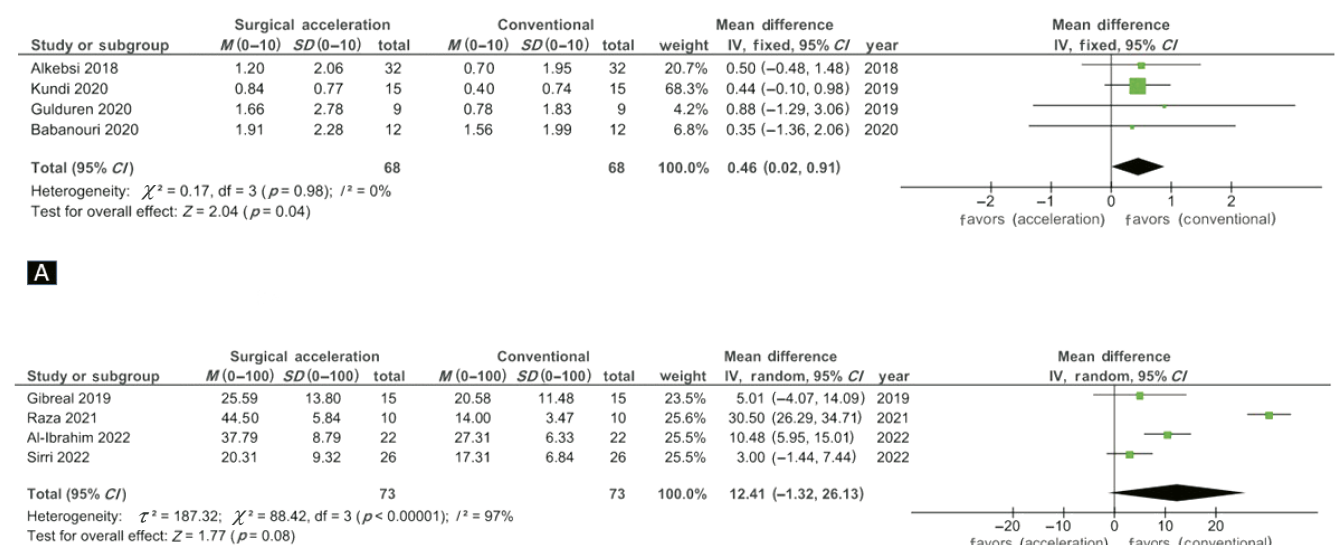

Fig. 3. Forest plot depicting the mean difference between surgical acceleration and conventional orthodontics for pain perception on assessment scales 1–10 (A) and on assessment scales 1–100 (B)
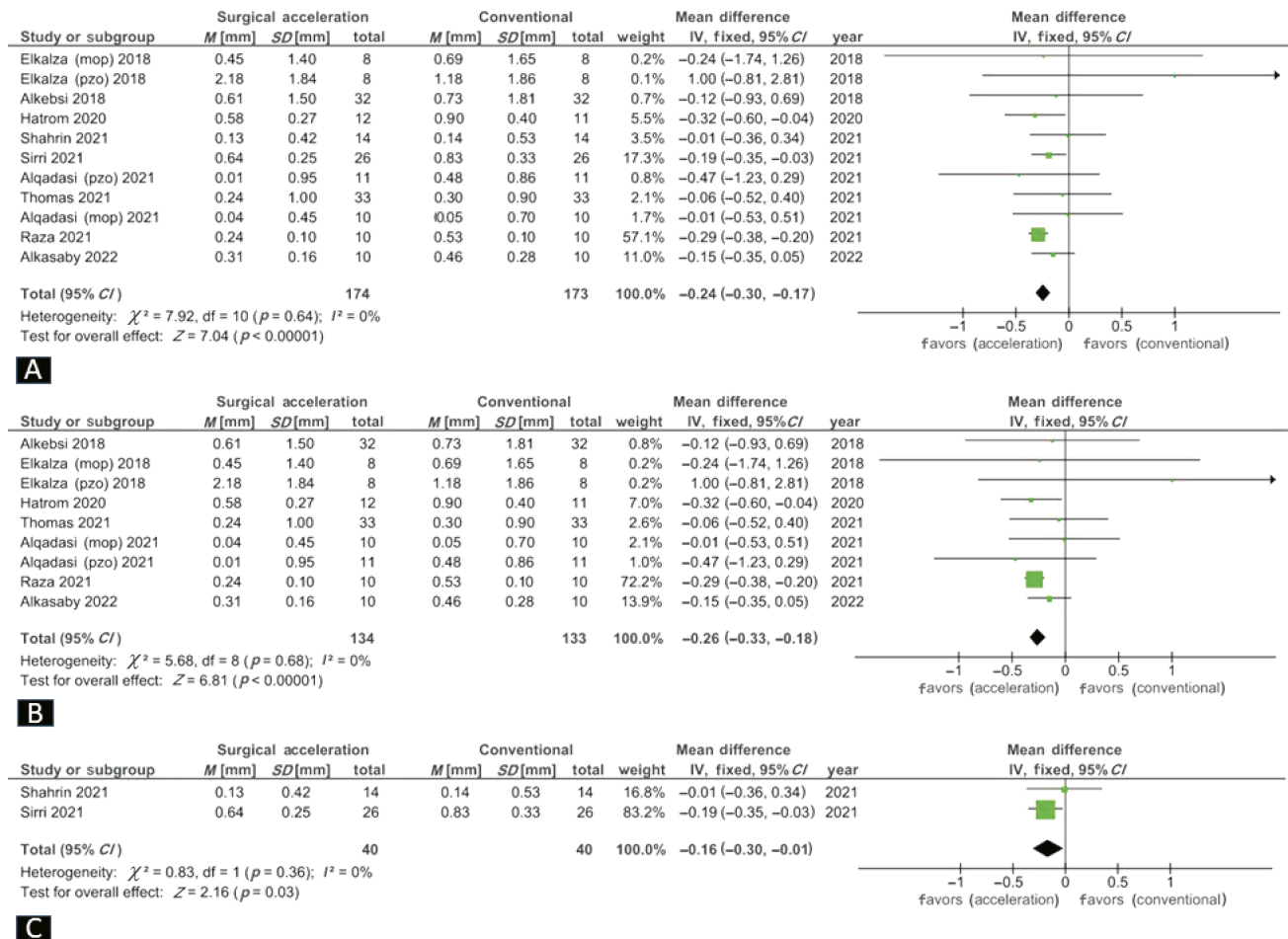
Fig. 4. Forest plot depicting the mean difference between surgical acceleration and conventional orthodontics for root resorption (A); in retraction (B) and in alignment (C)

The findings of this meta-analysis showed that surgical interventions together produced a greater perception of pain at 24 h, recorded on a scale of 0–10. The difference with regard to conventional treatment became non-significant when the analysis was performed at 7 days and on a scale of 0–100. Fu et al.[8] and MacDonald et al.[10] performed systematic reviews to assess pain perception in studies using acceleration surgery, but without quantitative analyses.

Dab et al.[9] and Mousa et al.[13] carried out meta-analyses to compare surgical interventions vs. conventional treatment with respect to pain perception without finding significant differences. However, some factors must be considered. Dab et al. included 2 studies that used periodontal accelerated osteogenic orthodontics (PAOO) and micro-osteoperforation, with the same units of measurement, but with unspecified follow-up time, and with the use of bone graft in one of the studies.[9] Mousa et al. analyzed 2 RCTs that also evaluated micro-osteoperforation with the same measurement scale and follow-up time, but only in canine retraction movements.[13] Although they found no differences, a similar trend was observed, which may become significant with a larger number of studies. In addition, it should be considered that the present study

did not only include minimally invasive surgical interventions. The hyperalgesia described in the study can also be attributed to a cascade of inflammatory mediators, such as bradykinin, histamine, serotonin, and substance P, released by the action of prostaglandin E2 (PGE2) and the RANK/RANKL pathway as the first inflammatory messengers in osteoclastogenesis.[6,55] No differences were found between surgical interventions and conventional treatment in over a 7-day period, perhaps due to advancement toward a less invasive approach, where variations in the production of biochemical mediators associated with mild or moderate initial pain tend to decrease with postoperative time, even from the first day.[33] In addition, it is worth mentioning that pain recording was self-reported and might be subject to decreased sensory memory, which is observed at a longer evaluation period.[25]

With respect to the root resorption outcome, it was found that surgical interventions produced significantly less resorption in general. A different result was found by Dab et al., who performed a meta-analysis of 4 studies that reported their results in linear millimeters and cubic millimeters, with different follow-up times from one another.[9] The authors concluded that there was no significant difference in root resorption between patients who

received any acceleration surgery and those who underwent conventional treatment.[9] However, the difference between both systematic reviews can be explained based on the criteria used in the present investigation, where only studies that reported the outcome in linear millimeters were considered. Although measurements made by cone beam computed tomography (CBCT) are more accurate, progress periapical radiographs are still the main method used to detect root resorption during treatment.[11] Furthermore, most of the studies that recorded measurements in cubic millimeters did not report complete quantitative data, or the follow-up period was insufficient. Consequently, a minimum acceptable follow-up of 3 months was established according to the literature.[7,11]

Acceleration surgeries caused less root resorption, which is partly due to the localized increase in the number of osteoclasts, which allowed a higher rate of movement with less root resorption.[5,6] However, there is evidence that the recruitment of factors like catabolic agents for remodeling can have an indiscriminate and deleterious effect on the surrounding tissues, e.g., cementum, ultimately depending on other factors, such as the application of optimal force.[12,32,56]

Finally, it is precisely the concept of the optimal application of force that can help explain the differences found between retraction and alignment movements. Although in both meta-analyses significantly less resorption was observed after acceleration surgeries as compared to conventional treatment, the difference was smaller in alignment movements, where the forces released may be less controlled with respect to retraction. In this sense, it should be considered that intrusion, retraction and torque movements by themselves may not be responsible for increasing the risk of resorption,[57] while the area of stress distribution, and the amount and lack of control of the force can play an important role in the exacerbation of root resorption in acceleration surgeries.[6,56]

Limitations

Among the main limitations are the deficiencies in reporting the results in the included studies, which precluded the inclusion of a greater number of investigations in the quantitative analysis. Although the number of participants was small in most investigations, this could have been offset by the number of investigations that were able to be included to maintain adequate power in the meta-analysis. It should be considered that only half of the trials included in the meta-analyses were assessed to have a low risk of bias with considerable heterogeneity, and this made it difficult to draw definitive conclusions. Future studies are needed, assessing not only the tooth movement rate, but also other patient-reported outcomes that could not be evaluated in the present systematic review, such as functional impairment (swelling, chewing, discomfort, mouth opening), the periodontal status and dental vitality.

Conclusions

Surgical interventions for the acceleration of tooth movement produced a greater perception of pain than conventional orthodontic treatment at 24 h of follow-up. However, the perception of pain was similar when it was evaluated after a period of 7 days.

Overall, there was evidence of significantly less root resorption in patients who received acceleration surgery for tooth movement in comparison with those who received conventional orthodontic treatment alone. Lower root resorption was also found when acceleration interventions were performed in tooth alignment movements, with a greater difference in retraction movements.

Ethics approval and consent to participate

Not applicable.

Data availability

All the data generated according to the objectives and methodology is published here. Any additional information can be obtained by contacting the corresponding author by e-mail.

Consent for publication

Not applicable.

ORCID iDs

Mariano Ortiz-Pizarro ⬤ https://orcid.org/0000-0002-3472-9997
Marcos Jimmy Carruitero-Honores
  ⬤ https://orcid.org/0000-0001-8339-5076
Silvio Augusto Bellini-Pereira ⬤ https://orcid.org/0000-0002-7785-1634
Aron Aliaga-Del Castillo ⬤ https://orcid.org/0000-0003-3963-1742

References

1. Yavuz MC, Sunar O, Buyuk SK, Kantarcı A. Comparison of piezocision and discision methods in orthodontic treatment. *Prog Orthod.* 2018;19(1):44. doi:10.1186/s40510-018-0244-y
2. Omidkhoda M, Radvar M, Azizi M, Dehghani M. Evaluating the efficacy of a modified piezo-puncture method on the rate of tooth movement in orthodontic patients: A clinical study. *Turk J Orthod.* 2020;33(1):13–20. doi:10.5152/TurkJOrthod.2020.19013
3. Al-Ibrahim HM, Hajeer MY, Burhan AS, Sultan K, Ajaj MA, Mahaini L. The efficacy of accelerating orthodontic tooth movement by combining self-ligating brackets with one or more acceleration methods: A systematic review. *Cureus.* 2022;14(12):e32879. doi:10.7759/cureus.32879
4. Khlef HN, Hajeer MY, Ajaj MA, Heshmeh O. En-masse retraction of upper anterior teeth in adult patients with maxillary or bimaxillary dentoalveolar protrusion: A systematic review and meta-analysis. *J Contemp Dent Pract.* 2019;20(1):113–127. PMID:31058623.
5. Alikhani M, Nervina JM, Teixeira CC. Chapter 17: Precision accelerated orthodontics. In: Krishnan V, Kuijpers-Jagtman AM, Davidovitch Z, eds. *Biological Mechanisms of Tooth Movement.* 3rd ed. Hoboken, NJ: John Wiley & Sons Ltd.; 2021:265–275. https://onlinelibrary.wiley.com/doi/abs/10.1002/9781119608912.ch17. Accessed August 19, 2022.
6. Yamaguchi M, Fukasawa S. Is inflammation a friend or foe for orthodontic treatment?: Inflammation in orthodontically induced inflammatory root resorption and accelerating tooth movement. *Int J Mol Sci.* 2021;22(5):2388. doi:10.3390/ijms22052388

7. Nieto-Nieto N, Solano JE, Yañez-Vico R. External apical root resorption concurrent with orthodontic forces: The genetic influence. *Acta Odontol Scand*. 2017;75(4):280–287. doi:10.1080/00016357.2017.1294260

8. Fu T, Liu S, Zhao H, Cao M, Zhang R. Effectiveness and safety of minimally invasive orthodontic tooth movement acceleration: A systematic review and meta-analysis. *J Dent Res*. 2019;98(13):1469–1479. doi:10.1177/0022034519878412

9. Dab S, Chen K, Flores-Mir C. Short- and long-term potential effects of accelerated osteogenic orthodontic treatment: A systematic review and meta-analysis. *Orthod Craniofac Res*. 2019;22(2):61–68. doi:10.1111/ocr.12272

10. MacDonald L, Zanjir M, Lighvan NL, Da Costa BR, Suri S, Azarpazhooh A. Efficacy and safety of different interventions to accelerate maxillary canine retraction following premolar extraction: A systematic review and network meta-analysis. *Orthod Craniofac Res*. 2021;24(1):17–38. doi:10.1111/ocr.12409

11. Krishnan V. Root resorption with orthodontic mechanics: Pertinent areas revisited. *Aust Dent J*. 2017;62(Suppl 1):71–77. doi:10.1111/adj.12483

12. Paetyangkul A, Türk T, Elekdağ-Türk S, et al. Physical properties of root cementum: Part 16. Comparisons of root resorption and resorption craters after the application of light and heavy continuous and controlled orthodontic forces for 4, 8, and 12 weeks. *Am J Orthod Dentofacial Orthop*. 2011;139(3):e279–e284. doi:10.1016/j.ajodo.2010.07.021

13. Mousa MM, Hajeer MY, Burhan AS, Almahdi WH. Evaluation of patient-reported outcome measures (PROMs) during surgically-assisted acceleration of orthodontic treatment: A systematic review and meta-analysis. *Eur J Orthod*. 2022;44(6):622–635. doi:10.1093/ejo/cjac038

14. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 Statement: An updated guideline for reporting systematic reviews. *J Clin Epidemiol*. 2021;134:178–189. doi:10.1016/j.jclinepi.2021.03.001

15. Sterne JA, Savović J, Page MJ, et al. RoB 2: A revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019;366:l4898. doi:10.1136/bmj.l4898

16. Sterne JA, Hernán MA, Reeves BC, et al. ROBINS-I: A tool for assessing risk of bias in non-randomised studies of interventions. *BMJ*. 2016;355:i4919. doi:10.1136/bmj.i4919

17. Shoreibah EA, Salama AE, Attia MS, Al-Moutaseuem Abu-Seida SM. Corticotomy-facilitated orthodontics in adults using a further modified technique. *J Int Acad Periodontol*. 2012;14(4):97–104. PMID:23210198.

18. Alikhani M, Raptis M, Zoldan B, et al. Effect of micro-osteoperforations on the rate of tooth movement. *Am J Orthod Dentofacial Orthop*. 2013;144(5):639–648. doi:10.1016/j.ajodo.2013.06.017

19. Al-Naoum F, Hajeer MY, Al-Jundi A. Does alveolar corticotomy accelerate orthodontic tooth movement when retracting upper canines? A split-mouth design randomized controlled trial. *J Oral Maxillofac Surg*. 2014;72(10):1880–1889. doi:10.1016/j.joms.2014.05.003

20. Abbas NH, Sabet NE, Hassan IT. Evaluation of corticotomy-facilitated orthodontics and piezocision in rapid canine retraction. *Am J Orthod Dentofacial Orthop*. 2016;149(4):473–480. doi:10.1016/j.ajodo.2015.09.029

21. Patterson BM, Dalci O, Papadopoulou AK, et al. Effect of piezocision on root resorption associated with orthodontic force: A micro-computed tomography study. *Am J Orthod Dentofacial Orthop*. 2017;151(1):53–62. doi:10.1016/j.ajodo.2016.06.032

22. Alkebsi A, Al-Maaitah E, Al-Shorman H, Alhaija EA. Three-dimensional assessment of the effect of micro-osteoperforations on the rate of tooth movement during canine retraction in adults with Class II malocclusion: A randomized controlled clinical trial. *Am J Orthod Dentofacial Orthop*. 2018;153(6):771–785. doi:10.1016/j.ajodo.2017.11.026

23. Chan E, Dalci O, Petocz P, Papadopoulou AK, Darendeliler MA. Physical properties of root cementum: Part 26. Effects of micro-osteoperforations on orthodontic root resorption: A micro-computed tomography study. *Am J Orthod Dentofacial Orthop*. 2018;153(2):204–213. doi:10.1016/j.ajodo.2017.05.036

24. Elkalza AR, Hashem AS, Alam MK. Comparative study of root resorption between two methods for accelerated orthodontic tooth movement. *J Oral Res*. 2018;7(9):412–417. doi:10.17126/joralres.2018.086

25. Sivarajan S, Doss JG, Papageorgiou SN, Cobourne MT, Wey MC. Mini-implant supported canine retraction with micro-osteoperforation: A split-mouth randomized clinical trial. *Angle Orthod*. 2019;89(2):183–189. doi:10.2319/011518-47.1

26. Aboalnaga AA, Salah Fayed MM, El-Ashmawi NA, Soliman SA. Effect of micro-osteoperforation on the rate of canine retraction: A split-mouth randomized controlled trial. *Prog Orthod*. 2019;20(1):21. doi:10.1186/s40510-019-0274-0

27. Alqadasi B, Aldhorae K, Halboub E, et al. The effectiveness of micro-osteoperforations during canine retraction: A three-dimensional randomized clinical trial. *J Int Soc Prev Community Dent*. 2019;9(6):637–645. doi:10.4103/jispcd.JISPCD_233_19

28. Bansal M, Sharma R, Kumar D, Gupta A. Effects of mini-implant facilitated micro-osteoperforations in alleviating mandibular anterior crowding: A randomized controlled clinical trial. *J Orthod Sci*. 2019;8:19. doi:10.4103/jos.JOS_112_18

29. Charavet C, Lecloux G, Jackers N, Albert A, Lambert F. Piezocision-assisted orthodontic treatment using CAD/CAM customized orthodontic appliances: A randomized controlled trial in adults. *Eur J Orthod*. 2019;41(5):495–501. doi:10.1093/ejo/cjy082

30. Gibreal O, Hajeer MY, Brad B. Evaluation of the levels of pain and discomfort of piezocision-assisted flapless corticotomy when treating severely crowded lower anterior teeth: A single-center, randomized controlled clinical trial. *BMC Oral Health*. 2019;19(1):57. doi:10.1186/s12903-019-0758-9

31. Alfawal AMH, Hajeer MY, Ajaj MA, Hamadah O, Brad B, Latifeh Y. Evaluation of patient-centered outcomes associated with the acceleration of canine retraction by using minimally invasive surgical procedures: A randomized clinical controlled trial. *Dent Med Probl*. 2020;57(3):285–293. doi:10.17219/dmp/120181

32. Babanouri N, Ajami S, Salehi P. Effect of mini-screw-facilitated micro-osteoperforation on the rate of orthodontic tooth movement: A single-center, split-mouth, randomized, controlled trial. *Prog Orthod*. 2020;21(1):7. doi:10.1186/s40510-020-00306-8

33. Gulduren K, Tumer H, Oz U. Effects of micro-osteoperforations on intraoral miniscrew anchored maxillary molar distalization: A randomized clinical trial. *J Orofac Orthop*. 2020;81(2):126–141. doi:10.1007/s00056-019-00207-4

34. Hatrom AA, Zawawi KH, Al-Ali RM, et al. Effect of piezocision corticotomy on en-masse retraction. *Angle Orthod*. 2020;90(5):648–654. doi:10.2319/092719-615.1

35. Ibrahim AM, Ibrahim SA, Hassan SA, Abd el-Samad FA, Attia MS. Assessment of root changes and bone density accompanying different methods of accelerated orthodontic tooth movement. *Al-Azhar Dent J Girls*. 2020;7(4-A):627–633. doi:10.21608/ADJG.2020.13117.1148

36. Kundi I, Alam MK, Shaheed S. Micro-osteo perforation effects as an intervention on canine retraction. *Saudi Dent J*. 2020;32(1):15–20. doi:10.1016/j.sdentj.2019.05.009

37. Mahmoudzadeh M, Poormoradi B, Alijani S, Farhadian M, Kazemisaleh A. Efficacy of Er,Cr laser incision corticotomy in rapid maxillary canine retraction: A split-mouth randomized clinical trial. *J Lasers Med Sci*. 2020;11(4):442–449. doi:10.34172/jlms.2020.69

38. Raj SC, Praharaj K, Barik AK, et al. Retraction with and without piezocision-facilitated orthodontics: A randomized controlled trial. *Int J Periodontics Restorative Dent*. 2020;40(1):e19–e26. doi:10.11607/prd.3968

39. Alqadasi B, Xia HY, Alhammadi MS, Hasan H, Aldhorae K, Halboub E. Three-dimensional assessment of accelerating orthodontic tooth movement-micro-osteoperforations vs piezocision: A randomized, parallel-group and split-mouth controlled clinical trial. *Orthod Craniofac Res*. 2021;24(3):335–343. doi:10.1111/ocr.12437

40. Jaber ST, Al-Sabbagh R, Hajeer MY. Evaluation of the efficacy of laser-assisted flapless corticotomy in accelerating canine retraction: A split-mouth randomized controlled clinical trial. *Oral Maxillofac Surg*. 2022;26(1):81–89. doi:10.1007/s10006-021-00963-x

41. Ozkan TH, Arici S. The effect of different micro-osteoperforation depths on the rate of orthodontic tooth movement: A single-center, single-blind, randomized clinical trial. *Korean J Orthod*. 2021;51(3):157–165. doi:10.4041/kjod.2021.51.3.157

42. Ravi MS, Murali PS, Kumari V, Hamidi W, Sneha K, Soans C. Radiographic and histochemical assessment of root resorption during canine retraction through piezocision site. *Indian J Dent Res*. 2021;32(4):448–452. doi:10.4103/ijdr.IJDR_654_19

43. Raza M, Sharma P, Kumar P, Vaish S, Pathak B. Comparison of canine retraction by conventional and corticotomy-facilitated methods: A split mouth clinical study. *J Orthod Sci*. 2021;10:9. doi:10.4103/jos.JOS_12_20

44. Shahrin AA, Abdul Ghani SH, Norman NH. Effect of micro-osteoperforations on external apical root resorption: A randomized controlled trial. *Korean J Orthod*. 2021;51(2):86–94. doi:10.4041/kjod.2021.51.2.86

45. Sirri MR, Burhan AS, Hajeer MY, Nawaya FR. Evaluation of corticision-based acceleration of lower anterior teeth alignment in terms of root resorption and dehiscence formation using cone-beam computed tomography in young adult patients: A randomized controlled trial. *Int Orthod*. 2021;19(4):580–590. doi:10.1016/j.ortho.2021.10.001

46. Thomas S, Das SK, Barik AK, Raj SC, Rajasekaran A, Mishra M. Evaluation of physiodispenser assisted micro-osteoperforation on the rate of tooth movement and associated periodontal tissue status during individual canine retraction in first premolar extraction cases: A split-mouth randomized controlled clinical trial. *J World Fed Orthod*. 2021;10(3):89–97. doi:10.1016/j.ejwf.2021.05.001

47. Alkasaby AA, Shamaa MS, Abdelnaby YL. The effects of micro-osteoperforation on upper first molar root resorption and bone density after distalization by miniscrew-supported Fast Back appliance in adults: A CBCT randomized controlled trial. *Int Orthod*. 2022;20(1):100611. doi:10.1016/j.ortho.2022.100611

48. Arana JG, Rey D, Ríos H, et al. Root resorption in relation to a modified piezocision technique. *Angle Orthod*. 2022;92(3):347–352. doi:10.2319/121520-1009.1

49. Hawkins VM, Papadopoulou AK, Wong M, Pandis N, Dalci O, Darendeliler MA. The effect of piezocision vs no piezocision on maxillary extraction space closure: A split-mouth, randomized controlled clinical trial. *Am J Orthod Dentofacial Orthop*. 2022;161(1):7–19.e2. doi:10.1016/j.ajodo.2021.06.015

50. Al-Ibrahim HM, Hajeer MY, Burhan AS, Alkhouri I, Latifeh Y. Evaluation of patient-centered outcomes associated with the acceleration of upper incisor decrowding using self-ligating brackets with or without piezocision in comparison with traditional brackets: A three-arm randomized controlled trial. *Cureus*. 2022;14(6):e26467. doi:10.7759/cureus.26467

51. Kumar P, Rampurawala AH, Patil AS. Effect of micro-osteoperforations (MOPs) on the rate of en masse orthodontic tooth retraction: A randomized controlled trial. *J Orofac Orthop*. 2024;85(3):189–198. doi:10.1007/s00056-022-00420-8

52. Li J, Papadopoulou AK, Gandedkar N, Dalci K, Darendeliler MA, Dalci O. The effect of micro-osteoperforations on orthodontic space closure investigated over 12 weeks: A split-mouth, randomized controlled clinical trial. *Eur J Orthod*. 2022;44(4):427–435. doi:10.1093/ejo/cjab079

53. Sirri MR, Burhan AS, Hajeer MY, Nawaya FR. Evaluation of perceived pain, discomfort, functional impairments, and satisfaction when relieving crowded lower anterior teeth in young adult patients using corticision-assisted fixed orthodontic treatment: A randomized controlled trial. *Cureus*. 2022;14(7):e26489. doi:10.7759/cureus.26489

54. Sultana S, Rahman NA, Akmar Zainuddin SL, Ahmad B. Effect of piezocision procedure in levelling and alignment stage of fixed orthodontic treatment: A randomized clinical trial. *Sci Rep*. 2022;12(1):6230. doi:10.1038/s41598-022-09851-0

55. Yuhan DM, Shetty S, Nambiar S, Philip N, Ashith MV. Accelerated orthodontics – surgical, mechanical and pharmacological methods. *Eur J Mol Clin Med*. 2020;7(10):1293–1306. https://ejmcm.com/uploads/paper/54be127d0c0fe0434732dab0c4f9e638.pdf. Accessed September 2, 2022.

56. Nanda A, Chen PJ, Mehta S, et al. The effect of differential force system and minimal surgical intervention on orthodontic tooth movement and root resorption. *Eur J Orthod*. 2021;43(6):607–613. doi:10.1093/ejo/cjaa065

57. Yassir YA, McIntyre GT, Bearn DR. Orthodontic treatment and root resorption: An overview of systematic reviews. *Eur J Orthod*. 2021;43(4):442–456. doi:10.1093/ejo/cjaa058

Review

Mechanical debridement combined with amoxicillin and metronidazole compared with mechanical debridement alone for the treatment of chronic periodontitis: An overview of systematic reviews

Daniela Zúñiga-Loor[1,A–F], Juan Marcos Parise-Vasco[2,3,B–F], Camila Montesinos-Guevara[3,A,C–F]

[1] Department of Periodontics and Surgical Implantology, "Eugenio Espejo" Faculty of Health Sciences, UTE University, Quito, Ecuador
[2] Department of Epidemiology and Applied Clinical Research, "Eugenio Espejo" Faculty of Health Sciences, UTE University, Quito, Ecuador
[3] Centro de Investigación en Epidemiología Clínica y Salud Pública (CISPEC), "Eugenio Espejo" Faculty of Health Sciences, UTE University, Quito, Ecuador

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

Abstract

Periodontal mechanical debridement is the most common therapy for the treatment of periodontitis. However, depending on the severity of the disease, mechanical debridement has been recommended in combination with systemic antibiotics. In this study, we performed an overview of systematic reviews using the Friendly Summaries of Body of Evidence using Epistemonikos (FRISBEE) methodology on the effectiveness and safety of mechanical debridement combined with amoxicillin and metronidazole compared to mechanical debridement alone for the treatment of chronic periodontitis. We conducted a systematic search of the Epistemonikos database, extracted data from 10 systematic reviews and re-analyzed data from 23 primary studies to generate a summary of findings (SoF) table. We used RevMan 5.3 and GRADEpro for data analysis and data presentation. The following outcomes were analyzed: probing depth (mean difference (MD): 0.07 mm); clinical attachment level (MD: 0.04 mm); bleeding on probing (MD: 5.06%); and suppuration (MD: 0.31%). There was no evidence of a clinically relevant benefit of periodontal mechanical debridement therapy combined with amoxicillin and metronidazole compared to periodontal mechanical debridement therapy alone for the treatment of chronic periodontitis in the studied periodontal outcomes.

Keywords: metronidazole, amoxicillin, periodontal debridement, periodontal diseases

Introduction

Oral diseases affect many people throughout their lives, causing pain, discomfort, deformity, and even death. They constitute a significant health burden for several countries.[1] The most common oral diseases are periodontal diseases, such as gingivitis and periodontitis, with a prevalence of up to 50% worldwide.[2] The absence of treatment can lead to tooth mobility and subsequent tooth loss,[3] which, depending on the severity of the disease, may affect a person's ability to chew and speak, and have a major impact on their quality of life.[4]

Periodontal treatment involves the removal of biofilms and microbial deposits from root surfaces to reduce the host inflammatory response and tissue degradation.[5] However, it is not possible to eliminate all pathogenic bacteria in the subgingival region using instrumentation alone; thus, antimicrobials are prescribed to eliminate the remaining subgingival microorganisms.[6] Systemically administered antibiotics reach periodontal tissues through the blood and attack microorganisms that are inaccessible to hand instruments.[7] They also help to eradicate infections by suppressing periodontal pathogens that invade subepithelial periodontal tissues and other extradental tissues, such as the deep crevices of the tongue.[6] Therefore, prescribing systemic antibiotics in therapy is considered beneficial for the eradication of pathogenic bacteria.[8]

In recent years, there has been a growing interest in the use of endogenous molecules or natural products as adjuvant therapies for the long-term treatment or maintenance of periodontitis.[9] However, antibiotic use is more common. Several combinations of systemic antibiotics are used with mechanical periodontal therapy, with amoxicillin and metronidazole being one of the most common.[10] Although this is a widespread practice, it is not yet clear how beneficial it is to prescribe them compared to performing mechanical periodontal therapy alone.[11] Several studies have indicated that the administration of antimicrobials in conjunction with mechanical debridement may have a beneficial effect on both the mechanical therapy and microbiological parameters.[6] The benefits of adjuvant therapy have been documented in several randomized controlled trials (RCTs), as well as in systematic reviews and meta-analyses.[6] Adjuvant therapies can help to reduce gingival inflammation, probing depth, attachment level loss, and the need for future periodontal surgery.[12]

The objective of the present study is to synthesize the available evidence regarding the practice of mechanical debridement in conjunction with amoxicillin and metronidazole compared to mechanical debridement alone for the treatment of chronic periodontitis. Additionally, we aim to report the therapeutic effectiveness of the aforementioned approaches, based on the outcomes of the studies, including the clinical attachment level, probing depth, radiographic bone loss, bleeding on probing, and suppuration.

Material and methods

An overview was conducted using the Friendly Summaries of Body of Evidence using Epistemonikos (FRISBEE) methodology[13] to synthesize the best available evidence on the practice of mechanical debridement combined with the prescription of amoxicillin and metronidazole for the treatment of chronic periodontitis. Our research question was based on the Population, Intervention, Comparison and Outcomes (PICO) strategy, as follows: population – patients with chronic periodontitis; intervention – mechanical debridement combined with amoxicillin and metronidazole; comparison – mechanical debridement; outcomes – probing depth, clinical attachment level, radiographic bone loss, bleeding on probing, and suppuration. Furthermore, a systematic search of the Epistemonikos database of systematic reviews, which synthesizes data from multiple sources, including MEDLINE, Embase, Cochrane Library, and LILACS, was performed.[14] No language restrictions were applied, and the search was limited to articles published until July 7, 2022.

The main search terms were "chronic periodontitis", "periodontal disease", "pyorrhoea", "pyorrhea", "amoxicillin", "amoxicilina", "amoxicilline", "amoxicillinum", "amoxycillin", "metronidazole", "flagyl", "fossyol", and "systemic antimicrobials". Duplicate articles were removed manually using Mendeley Desktop 1.19.8 software (https://www.mendeley.com/autoupdates/installers/1.19.8). Only systematic reviews that met the following eligibility criteria were included: studies in adult patients with chronic periodontitis who had received mechanical debridement combined with amoxicillin and metronidazole as an intervention or mechanical debridement alone as a comparison.

Two authors (DZL, JMPV) independently screened systematic reviews for eligibility based on their titles and abstracts, followed by a full-text screening. Primary studies from the systematic reviews that met the inclusion criteria were selected for data extraction. Any disagreement between the 2 authors was resolved through discussion and consensus, with the arbitration of the third author (CMG) when necessary. A matrix in the XLSX format was employed as an instrument for data collection using Microsoft Excel 2022 (Microsoft Corporation, Redmond, USA), which included the search strategy, characteristics of systematic reviews and primary studies, the assessment of the risk of bias for primary studies, and relevant outcomes.

RevMan 5.3 software (Cochrane Collaboration, London, UK) was used for data analysis, and GRADEpro software (GRADE Working Group) was used to assess the certainty of the evidence. In addition, we developed a summary of findings (SoF) table according to the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) approach. The data was entered

into RevMan v. 5.3 software. Regarding measures of treatment effect, we found only outcomes with continuous data. We combined the results by performing a meta-analysis for each outcome using a random-effects model with inverse-variance weighting. Results were reported as mean difference (*MD*), calculated with its 95% confidence interval (*CI*). The unit of analysis for all predefined outcomes was periodontal sites of therapeutic intervention. We assessed statistical heterogeneity in each meta-analysis using the Tau^2, I^2 and χ^2 statistics. We considered heterogeneity to be substantial if the I^2 was greater than 75% and either the Tau^2 was greater than 0 or there was a low *p*-value (<0.05) in the χ^2 test for heterogeneity.

To interpret the results and to rate the certainty of the evidence, we employed the GRADE approach and GRADEpro software. First, we analyzed the overall certainty of the evidence for each outcome individually, downgrading the evidence from "high certainty" to "moderate", "low" or "very low", depending on the risk of bias, indirectness of the evidence, inconsistency, and imprecision of the effect estimates. The research protocol is available from the corresponding author upon request.

Results

The systematic search yielded a total of 18 articles, of which 1 was a duplicate. The remaining 17 articles were screened based on their titles and abstracts, and 16 studies were included for full-text screening. Ten systematic reviews[6,11,15–22] were ultimately included in the development of this FRISBEE overview, which included 23 primary studies.[10,23–44] All primary studies were randomized clinical trials, reported in 27 references (Fig. 1).[10,23–48]

All studies included systemically healthy adult patients (18–70 years old) with a diagnosis of chronic periodontitis. The patients were required to have at least 4 teeth diagnosed with a probing depth greater than 4 mm, a clinical attachment level greater than or equal to 2 mm, and radiographic evidence of bone loss. Additionally, all trials compared the use of mechanical debridement combined with amoxicillin and metronidazole to mechanical debridement alone for the treatment of chronic periodontitis, with a minimum follow-up period of 3 months.

In 7 out of the 23 included studies, amoxicillin 375 mg and metronidazole 250 mg were administered.[10,23,29,31,38,43,44] In 5 studies, amoxicillin 500 mg and metronidazole 400 mg were administered together.[28,30,37,41,42] In 4 studies, amoxicillin 500 mg and metronidazole 500 mg were prescribed.[24,27,34,35] In 3 studies, amoxicillin 500 mg and metronidazole 250 mg were prescribed.[25,32,36] In 2 studies, amoxicillin 375 mg and metronidazole 500 mg were administered together.[26,39] In 1 study, amoxicillin 500 mg and metronidazole 200 mg were prescribed,[33] and in another study, amoxicillin 250 mg and metronidazole 200 mg were administered.[40]
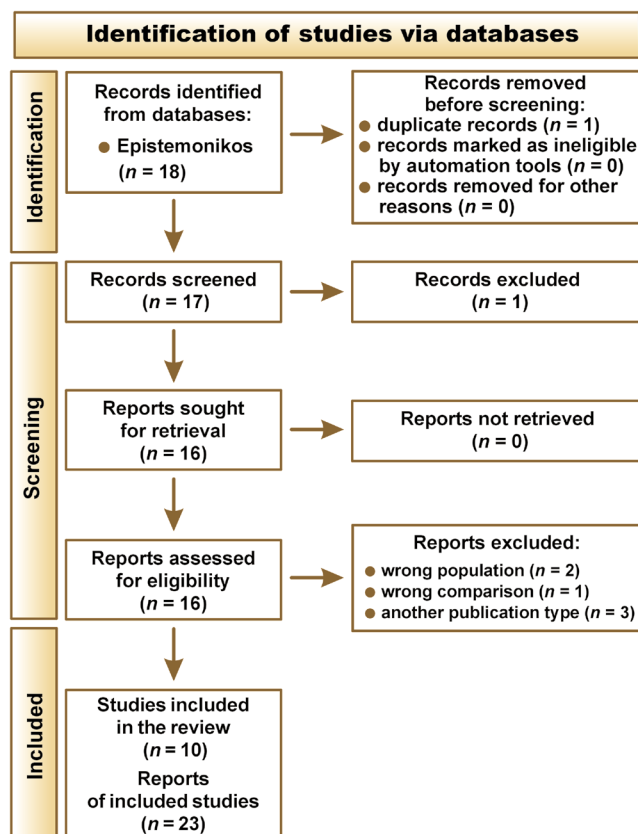

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart of systematic review selection process

Twenty-one trials measured probing depth (849 patients),[10,23–30,32–42,44] 18 trials assessed the clinical insertion level (758 patients),[10,23–30,32,34–37,40–42,44] 15 trials evaluated bleeding on probing (585 patients),[23,25–28,30,32–39,41] and 3 trials reported the percentage of patients with suppuration (142 patients).[30,37,41] There were no clinical trials reporting radiographic bone loss as an outcome (Table 1).

Based on the analysis performed with the included studies, the main findings of this overview are as follows (Table 1):
- periodontal mechanical debridement therapy combined with amoxicillin and metronidazole could decrease probing depth (*MD*: 0.07 mm; 95% *CI*: 0.20–0.05) and increase the clinical attachment level (*MD*: 0.04 mm; 95% *CI*: 0.14–0.21) compared with periodontal mechanical debridement therapy alone (low certainty of the evidence);
- radiographic bone loss was not measured or reported in any systematic review or primary article;
- it is uncertain whether periodontal mechanical debridement therapy combined with amoxicillin and metronidazole reduces the percentage of bleeding on probing and modifies the percentage of suppuration compared with periodontal mechanical debridement therapy alone (very low certainty of the evidence).

Table 1. Summary of findings (SoF) table using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach

Variable	Absolute effects		MD (95% CI)	Relative effect (95% CI)	Certainty of the evidence (GRADE)
	mechanical debridement alone	mechanical debridement in combination with amoxicillin and metronidazole			
Probing depth [mm]	3.20	3.13	−0.07 (−0.20–0.05)	–	⊕⊕◯◯ low[a,b]
Clinical attachment level [mm]	3.54	3.58	0.04 (−0.14–0.21)	–	⊕⊕◯◯ low[a,b]
Radiographic bone loss	not reported		–	–	not reported
Bleeding on probing [%]	37.00	31.94	−5.06 (−8.22––1.90)	–	⊕◯◯◯ very low[a,c,d,e]
Suppuration [%]	0.30	−0.01	−0.31 (−0.65–0.02)	–	⊕◯◯◯ very low[d,f,g]

CI – confidence interval; MD – mean difference; [a] certainty of the evidence was downgraded 1 level for risk of bias because the random sequence generation, allocation concealment, and blinding of participants or personnel were unclear in most of the included studies; in some trials, the missing outcome data was unclear; [b] certainty of the evidence was downgraded 1 level for imprecision because each end of the CI led to a different decision; [c] certainty of the evidence was downgraded 1 level due to inconsistency because the meta-analysis presented heterogeneity (I²: 94%); [d] certainty of the evidence was downgraded 1 level due to indirectness of the evidence, as it corresponded to a surrogate outcome; [e] certainty of the evidence was downgraded 1 level due to imprecision, as the CI for the magnitude of the effect was large; [f] certainty of the evidence was downgraded 1 level for risk of bias, as blinding of participants and personnel was unclear in 1 out of 3 trials; [g] certainty of the evidence was downgraded 1 level for imprecision, as the studies together had a small sample size. Low certainty of the evidence indicates that our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect. Very low certainty of the evidence indicates that we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect. An interactive version of this table is available at: https://gdt.gradepro.org/presentations/#/isof/isof_8c9dcdee-7cab-4f42-bf5f-03135aa86929-1657757367316.

Discussion

The results of this study are applicable to all systemically healthy adult patients over the age of 18 with mild or moderate chronic periodontitis. The objective of this study was to evaluate 5 outcomes. However, one of the proposed outcomes was not reported in the studies that met the eligibility criteria. Two of the remaining 4 outcomes were considered critical in the evaluation of periodontal parameters in periodontitis, namely probing depth and the clinical attachment level. Additionally, bleeding on probing and suppuration were evaluated as surrogate outcomes. The GRADE approach revealed that the evidence supporting the advantages of periodontal mechanical debridement therapy combined with amoxicillin and metronidazole compared to periodontal mechanical debridement therapy alone was of low or very low certainty.

The GRADE approach criteria revealed issues with the domains of risk of bias, inconsistency and imprecision. In several trials, the process of random sequence generation, allocation concealment, and blinding of participants and assessors was unclear.[23,32,34,36,44] Additionally, there was missing outcome data when assessing the risk of bias,[36,38] and the reasons why data or records were lost or unavailable were not stated, which may introduce bias in the estimation of the effect of the intervention. With regard to the inconsistency domain, high heterogeneity was observed in the magnitude of the effect on bleeding outcomes, with an I² of 94%.[10,23,25–28,30,32–38,40,41] Regarding imprecision, the effect of bleeding on probing outcomes had a wide CI, making it difficult to assess the magnitude

of the effect in a single clinical trial. In addition, the limits of the CIs for probing depth and clinical insertion level outcomes led us to a different decision regarding the effectiveness of periodontal mechanical debridement therapy combined with amoxicillin and metronidazole compared to periodontal mechanical debridement therapy alone. However, a strength of this study was the achievement of an optimal sample size for the outcomes of probing depth and the clinical insertion level (849 and 758 patients, respectively).

The results of our study were consistent with 2 of the identified systematic reviews,[6,15] which found no clinically relevant effect on any of the periodontal parameters studied when comparing the effectiveness of periodontal mechanical debridement therapy combined with amoxicillin and metronidazole to mechanical periodontal therapy alone for the treatment of chronic periodontitis. In both studies, the certainty of the evidence was also very low. However, our results are in disagreement with the reviews performed by Zandbergen et al., Herrera et al. and Haffajee et al., which indicate that probing depth, the clinical insertion level and bleeding on probing improved in the antibiotic group.[18,19,22] Despite the positive results of these studies regarding periodontal outcomes when antibiotics were administered, none of the studies established whether these results are clinically relevant. Therefore, it is not reported whether only a favorable statistical result was obtained or whether this minimal favorable difference represents a significant benefit to patients. In addition, our study partially aligns with 3 reviews that identified an additional benefit in probing depth and insertion level outcomes, but not in bleeding on probing.[11,16,20,21]

Likewise, our results are in partial agreement with the review performed by Santos et al., who observed positive periodontal outcomes in patients who received amoxicillin and metronidazole. However, due to the limited evidence, it was not possible to determine whether the adjunctive use of antibiotics provided a greater benefit than mechanical periodontal therapy alone.[17]

Although the combination of amoxicillin and metronidazole with periodontal mechanical debridement therapy may improve certain periodontal clinical parameters, bacterial resistance and adverse effects of antibiotics should also be considered.[18] Over the past 3 decades, antimicrobial resistance among microorganisms has increased steadily, and the susceptibility of bacteria to antimicrobial agents has become less predictable.[6] For this reason, the use of antibiotics in periodontitis should be restricted to specific cases in which the condition is severe or the patient carries a high risk of disease progression and has not responded to conventional mechanical periodontal therapy.[18]

The European Federation of Periodontology (EFP) has indicated in a clinical practice guideline that although there is evidence that antibiotics systemically improve the clinical outcome of subgingival instrumentation, their routine use in therapy is not recommended, except in cases of generalized periodontitis stage III, which involves a deep probing depth of ≥6 mm or complex anatomical surfaces (root concavities, furcations, infra bony pockets) that may have a difficult resolution. In such cases, the endpoints of mechanic therapy may not be achieved, and further treatment might need to be implemented.[49] Likewise, the UK version of the EFP S3-level clinical practice guideline recommends that the use of systemic antibiotics should not be considered a routine adjunct to subgingival instrumentation in patients with periodontitis. However, it should be considered an adjunct specifically in patients with severe generalized periodontitis.[50] On the other hand, the American Dental Association's clinical practice guideline on non-surgical treatment of chronic periodontitis indicates that in patients with moderate to severe chronic periodontitis, clinicians may consider systemic antimicrobials as an adjunct to mechanical therapy, with little expected net benefit, only after other alternatives have been considered.[51]

The keywords described in the methods section were used to identify 20 ongoing RCTs on this topic, from 2014 to 2022, in the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP) and in ClinicalTrials.gov.[52–71] Furthermore, we identified 11 ongoing systematic reviews in the National Institute for Health Research (NIHR) International Prospective Register of Systematic Reviews (PROSPERO),[72–82] regarding the administration of amoxicillin and metronidazole in periodontal mechanical debridement therapy for the treatment of chronic periodontitis. In the future, these reviews could provide relevant evidence on the clinical question raised in this study.

The main strength of this study is the applied methodology, which involved an in-depth literature search and the synthesis of all available evidence from systematic reviews and primary studies on the practice of mechanical debridement combined with amoxicillin and metronidazole for the treatment of chronic periodontitis, resulting in the identification of 23 primary studies.

Limitations

A limitation of this study is the high variability in the doses of amoxicillin and metronidazole when used as adjuvant therapy. This variability ranged from amoxicillin 250 mg and metronidazole 200 mg to amoxicillin 200 mg and metronidazole 500 mg. Another drawback observed in several clinical trials was the limitations in the randomization sequence generation, concealment, and blinding of participants or personnel. It is recommended that future clinical trials on this topic address this deficiency in their protocols before the start of the study, thus improving the certainty of the currently available evidence. Additionally, the majority of studies classified periodontal diseases based on the old classification by Armitage,[83] which limited comparisons between studies. Future clinical trials should classify periodontal diseases based on new classifications, such as the World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions,[84] which was released by the American Academy of Periodontology and the EFP. Such an approach would facilitate the interpretation of the results. Furthermore, it is recommended that further secondary research be conducted to evaluate the effectiveness and a risk–benefit ratio of the use of other systemic antibiotics complementary to periodontal mechanical debridement therapy.

Conclusions

This study found no evidence of a clinically relevant benefit of periodontal mechanical debridement therapy combined with amoxicillin and metronidazole compared to periodontal mechanical debridement therapy alone for the treatment of chronic periodontitis when analyzing periodontal parameters such as probing depth, the clinical attachment level, bleeding on probing, and suppuration. However, the confidence in the effect is limited due to a low and very low certainty of the evidence for the included outcomes. Although none of the systematic reviews reported on bacterial resistance and the adverse effects of antibiotics, we consider these outcomes to be relevant to limiting antibiotic use in daily clinical practice.

Ethics approval and consent to participate

Not applicable.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Daniela Zúñiga-Loor   https://orcid.org/0000-0002-9034-5920
Juan Marcos Parise-Vasco   https://orcid.org/0000-0002-5223-3370
Camila Montesinos-Guevara   https://orcid.org/0000-0001-6763-3644

References

1. Peres MA, Macpherson LMD, Weyant RJ, et al. Oral diseases: A global public health challenge. *Lancet*. 2019;394(10194):249–260. doi:10.1016/S0140-6736(19)31146-8
2. Chen MX, Zhong YJ, Dong QQ, Wong HM, Wen YF. Global, regional, and national burden of severe periodontitis, 1990–2019: An analysis of the Global Burden of Disease Study 2019. *J Clin Periodontol*. 2021;48(9):1165–1188. doi:10.1111/jcpe.13506
3. Helal O, Göstemeyer G, Krois J, Fawzy El Sayed K, Graetz C, Schwendicke F. Predictors for tooth loss in periodontitis patients: Systematic review and meta-analysis. *J Clin Periodontol*. 2019;46(7):699–712. doi:10.1111/jcpe.13118
4. Uy SNMR, Deng K, Fok CTC, Fok MR, Pelekos G, Tonetti MS. Food intake, masticatory function, tooth mobility, loss of posterior support, and diminished quality of life are associated with more advanced periodontitis stage diagnosis. *J Clin Periodontol*. 2022;49(3):240–250. doi:10.1111/jcpe.13588
5. Munasur SL, Turawa EB, Chikte UME, Musekiwa A. Mechanical debridement with antibiotics in the treatment of chronic periodontitis: Effect on systemic biomarkers – A systematic review. *Int J Environ Res Public Health*. 2020;17(15):5601. doi:10.3390/ijerph17155601
6. Khattri S, Kumbargere Nagraj S, Arora A, et al. Adjunctive systemic antimicrobials for the non-surgical treatment of periodontitis. *Cochrane Database Syst Rev*. 2020;11(11):CD012568. doi:10.1002/14651858.CD012568.pub2
7. Pretzl B, Sälzer S, Ehmke B, et al. Administration of systemic antibiotics during non-surgical periodontal therapy – A consensus report. *Clin Oral Investig*. 2019;23(7):3073–3085. doi:10.1007/s00784-018-2727-0
8. Kapoor A, Malhotra R, Grover V, Grover D. Systemic antibiotic therapy in periodontics. *Dent Res J (Isfahan)*. 2012;9(5):505–515. doi:10.4103/1735-3327.104866
9. Balaji TM, Varadarajan S, Jagannathan R, et al. Melatonin as a topical/systemic formulation for the management of periodontitis: A systematic review. *Materials (Basel)*. 2021;14(9):2417. doi:10.3390/ma14092417
10. Ribeiro ÉDP, Bittencourt S, Zanin ICJ, et al. Full-mouth ultrasonic debridement associated with amoxicillin and metronidazole in the treatment of severe chronic periodontitis. *J Periodontol*. 2009;80(8):1254–1264. doi:10.1902/JOP.2009.080403
11. Sgolastra F, Petrucci A, Ciarrocchi I, Masci C, Spadaro A. Adjunctive systemic antimicrobials in the treatment of chronic periodontitis: A systematic review and network meta-analysis. *J Periodontal Res*. 2021;56(2):236–248. doi:10.1111/JRE.12821
12. Walters J, Lai PC. Should antibiotics be prescribed to treat chronic periodontitis? *Dent Clin North Am*. 2015;59(4):919–933. doi:10.1016/j.cden.2015.06.011
13. Dallaserra M, Moreno B, Muñoz M, Cuellar J, Villanueva J. Summary of the body of evidence (FRISBEE). *Rev Clin Periodoncia Implantol Rehabil Oral*. 2019;12(2):113–115. doi:10.4067/S0719-01072019000200113
14. Verdugo-Paiva F, Zambrano-Achig P, Simancas-Racines D, Viteri-García A. Selective removal compared to complete removal for deep carious lesions. *Medwave*. 2020;20(1):e7758. doi:10.5867/MEDWAVE.2020.01.7758

15. Bono A, Brunotto M. Amoxicillin/metronidazole or scaling and root planing in the treatment of chronic periodontitis. *Acta Odontol Latinoam*. 2010;23(3):196–203. PMID:21638959.
16. Keestra JAJ, Grosjean I, Coucke W, Quirynen M, Teughels W. Non-surgical periodontal therapy with systemic antibiotics in patients with untreated chronic periodontitis: A systematic review and meta-analysis. *J Periodontal Res*. 2015;50(3):294–314. doi:10.1111/JRE.12221
17. Santos RS, Macedo RF, Souza EA, Soares RSC, Feitosa DS, Sarmento CFM. The use of systemic antibiotics in the treatment of refractory periodontitis: A systematic review. *J Am Dent Assoc*. 2016;147(7):577–585. doi:10.1016/J.ADAJ.2016.02.013
18. Zandbergen D, Slot DE, Niederman R, Van der Weijden FA. The concomitant administration of systemic amoxicillin and metronidazole compared to scaling and root planing alone in treating periodontitis: A systematic review. *BMC Oral Health*. 2016;16:27. doi:10.1186/s12903-015-0123-6
19. Herrera D, Sanz M, Jepsen S, Needleman I, Roldán S. A systematic review on the effect of systemic antimicrobials as an adjunct to scaling and root planing in periodontitis patients. *J Clin Periodontol*. 2002;29 Suppl 3:136–159. doi:10.1034/J.1600-051X.29.S3.8.X
20. Sgolastra F, Gatto R, Petrucci A, Monaco A. Effectiveness of systemic amoxicillin/metronidazole as adjunctive therapy to scaling and root planing in the treatment of chronic periodontitis: A systematic review and meta-analysis. *J Periodontol*. 2012;83(10):1257–1269. doi:10.1902/JOP.2012.110625
21. Garcia Canas P, Khouly I, Sanz J, Loomer PM. Effectiveness of systemic antimicrobial therapy in combination with scaling and root planing in the treatment of periodontitis: A systematic review. *J Am Dent Assoc*. 2015;146(3):150–163. doi:10.1016/J.ADAJ.2014.12.015
22. Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy. A systematic review. *Ann Periodontol*. 2003;8(1):115–181. doi:10.1902/ANNALS.2003.8.1.115
23. Berglundh T, Krok L, Liljenberg B, Westfelt E, Serino G, Lindhe J. The use of metronidazole and amoxicillin in the treatment of advanced periodontal disease. A prospective, controlled clinical trial. *J Clin Periodontol*. 1998;25(5):354–362. doi:10.1111/J.1600-051X.1998.TB02455.X
24. Boia S, Boariu M, Baderca F, et al. Clinical, microbiological and oxidative stress evaluation of periodontitis patients treated with two regimens of systemic antibiotics, adjunctive to non-surgical therapy: A placebo-controlled randomized clinical trial. *Exp Ther Med*. 2019;18(6):5001–5015. doi:10.3892/ETM.2019.7856
25. Borges I, Faveri M, Figueiredo LC, et al. Different antibiotic protocols in the treatment of severe chronic periodontitis: A 1-year randomized trial. *J Clin Periodontol*. 2017;44(8):822–832. doi:10.1111/JCPE.12721
26. Cionca N, Giannopoulou C, Ugolotti G, Mombelli A. Amoxicillin and metronidazole as an adjunct to full-mouth scaling and root planing of chronic periodontitis. *J Periodontol*. 2009;80(3):364–371. doi:10.1902/JOP.2009.080540
27. Cosgarea R, Juncar R, Heumann C, et al. Non-surgical periodontal treatment in conjunction with 3 or 7 days systemic administration of amoxicillin and metronidazole in severe chronic periodontitis patients. A placebo-controlled randomized clinical study. *J Clin Periodontol*. 2016;43(9):767–777. doi:10.1111/JCPE.12559
28. Dukić S, Matijević S, Daković D, Cutović T. Comparison of cefixime and amoxicillin plus metronidazole in the treatment of chronic periodontitis. *Vojnosanit Pregl*. 2016;73(6):526–530. doi:10.2298/VSP150326133D
29. Ehmke B, Beikler T, Haubitz I, Karch H, Flemmig TF. Multifactorial assessment of predictors for prevention of periodontal disease progression. *Clin Oral Investig*. 2003;7(4):217–221. doi:10.1007/S00784-003-0227-2
30. Feres M, Soares GMS, Mendes JAV, et al. Metronidazole alone or with amoxicillin as adjuncts to non-surgical treatment of chronic periodontitis: A 1-year double-blinded, placebo-controlled, randomized clinical trial. *J Clin Periodontol*. 2012;39(12):1149–1158. doi:10.1111/JCPE.12004
31. Flemmig TF, Milián E, Karch H, Klaiber B. Differential clinical treatment outcome after systemic metronidazole and amoxicillin in patients harboring *Actinobacillus actinomycetemcomitans* and/or *Porphyromonas gingivalis*. *J Clin Periodontol*. 1998;25(5):380–387. doi:10.1111/J.1600-051X.1998.TB02459.X

32. Goodson JM, Haffajee AD, Socransky SS, et al. Control of periodontal infections: A randomized controlled trial I. The primary outcome attachment gain and pocket depth reduction at treated sites. *J Clin Periodontol.* 2012;39(6):526–536. doi:10.1111/J.1600-051X.2012.01870.X

33. Li Y, Xu L, Lu RF, et al. Clinical effect of different sequences of debridement-antibiotic therapy in treatment of severe chronic periodontitis [in Chinese]. *Beijing Da Xue Xue Bao Yi Xue Ban.* 2015;47(1):27–31. PMID:25686324.

34. Liaw A, Miller C, Nimmo A. Comparing the periodontal tissue response to non-surgical scaling and root planing alone, adjunctive azithromycin, or adjunctive amoxicillin plus metronidazole in generalized chronic moderate-to-severe periodontitis: A preliminary randomized controlled trial. *Aust Dent J.* 2019;64(2):145–152. doi:10.1111/ADJ.12674

35. López NJ, Gamonal JA, Martinez B. Repeated metronidazole and amoxicillin treatment of periodontitis. A follow-up study. *J Periodontol.* 2000;71(1):79–89. doi:10.1902/JOP.2000.71.1.79

36. Moeintaghavi A, Reza Talebi-Ardakani M, Haerian-Ardakani A, et al. Adjunctive effects of systemic amoxicillin and metronidazole with scaling and root planing: A randomized, placebo controlled clinical trial. *J Contemp Dent Pract.* 2007;8(5):51–59. PMID:17618330.

37. Matarazzo F, Figueiredo LC, Braga Cruz SE, Faveri M, Feres M. Clinical and microbiological benefits of systemic metronidazole and amoxicillin in the treatment of smokers with chronic periodontitis: A randomized placebo-controlled study. *J Clin Periodontol.* 2008;35(10):885–896. doi:10.1111/J.1600-051X.2008.01304.X

38. Mombelli A, Brochut P, Plagnat D, Casagni F, Giannopoulou C. Enamel matrix proteins and systemic antibiotics as adjuncts to non-surgical periodontal treatment: Clinical effects. *J Clin Periodontol.* 2005;32(3):225–230. doi:10.1111/J.1600-051X.2005.00664.X

39. Mombelli A, Cionca N, Almaghlouth A, Décaillet F, Courvoisier DS, Giannopoulou C. Are there specific benefits of amoxicillin plus metronidazole in *Aggregatibacter actinomycetemcomitans*-associated periodontitis? Double-masked, randomized clinical trial of efficacy and safety. *J Periodontol.* 2013;84(6):715–724. doi:10.1902/JOP.2012.120281

40. Rooney J, Wade WG, Sprague SV, Newcombe RG, Addy M. Adjunctive effects to non-surgical periodontal therapy of systemic metronidazole and amoxycillin alone and combined. A placebo controlled study. *J Clin Periodontol.* 2002;29(4):342–350. doi:10.1034/J.1600-051X.2002.290410.X

41. Silva MP, Feres M, Oliveira Sirotto TA, et al. Clinical and microbiological benefits of metronidazole alone or with amoxicillin as adjuncts in the treatment of chronic periodontitis: A randomized placebo-controlled clinical trial. *J Clin Periodontol.* 2011;38(9):828–837. doi:10.1111/J.1600-051X.2011.01763.X

42. Soares GMS, Mendes JAV, Silva MP, et al. Metronidazole alone or with amoxicillin as adjuncts to non-surgical treatment of chronic periodontitis: A secondary analysis of microbiological results from a randomized clinical trial. *J Clin Periodontol.* 2014;41(4):366–376. doi:10.1111/JCPE.12217

43. van Winkelhoff AJ, Tijhof CJ, de Graaff J. Microbiological and clinical results of metronidazole plus amoxicillin therapy in *Actinobacillus actinomycetemcomitans*-associated periodontitis. *J Periodontol.* 1992;63(1):52–57. doi:10.1902/JOP.1992.63.1.52

44. Winkel EG, Van Winkelhoff AJ, Timmerman MF, Van der Velden U, Van der Weijden GA. Amoxicillin plus metronidazole in the treatment of adult periodontitis patients. A double-blind placebo-controlled study. *J Clin Periodontol.* 2001;28(4):296–305. doi:10.1034/J.1600-051X.2001.028004296.X

45. Cosgarea R, Heumann C, Juncar R, et al. One year results of a randomized controlled clinical study evaluating the effects of non-surgical periodontal therapy of chronic periodontitis in conjunction with three or seven days systemic administration of amoxicillin/metronidazole. *PLoS One.* 2017;12(6):e0179592. doi:10.1371/JOURNAL.PONE.0179592

46. Cionca N, Giannopoulou C, Ugolotti G, Mombelli A. Microbiologic testing and outcomes of full-mouth scaling and root planing with or without amoxicillin/metronidazole in chronic periodontitis. *J Periodontol.* 2010;81(1):15–23. doi:10.1902/JOP.2009.090390

47. Ehmke B, Moter A, Beikler T, Milian E, Flemmig TF. Adjunctive antimicrobial therapy of periodontitis: Long-term effects on disease progression and oral colonization. *J Periodontol.* 2005;76(5):749–759. doi:10.1902/JOP.2005.76.5.749

48. Giannopoulou C, Andersen E, Brochut P, Plagnat D, Mombelli A. Enamel matrix derivative and systemic antibiotics as adjuncts to non-surgical periodontal treatment: Biologic response. *J Periodontol.* 2006;77(4):707–713. doi:10.1902/JOP.2006.050166

49. Sanz M, Herrera D, Kebschull M, et al. Treatment of stage I–III periodontitis – The EFP S3 level clinical practice guideline. *J Clin Periodontol.* 2020;47 Suppl 22(Suppl 22):4–60. doi:10.1111/JCPE.13290

50. West N, Chapple I, Claydon N, et al. BSP implementation of European S3 – Level evidence-based treatment guidelines for stage I–III periodontitis in UK clinical practice. *J Dent.* 2021;106:103562. doi:10.1016/J.JDENT.2020.103562

51. Smiley CJ, Tracy SL, Abt E, et al. Evidence-based clinical practice guideline on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. *J Am Dent Assoc.* 2015;146(7):525–535. doi:10.1016/j.adaj.2015.01.026

52. Rabienejad N, Moradi Haghou J. Effect of ciprofloxacin versus amoxicillin and metronidazole in recovery of chronic periodontitis. https://trialsearch.who.int/Trial2.aspx?TrialID=IRCT2014010816141N1. Accessed July 14, 2022.

53. Moradi Haghgou J. Effect of amoxicillin and metronidazole in recovery of chronic periodontitis. https://trialsearch.who.int/Trial2.aspx?TrialID=IRCT2014020816141N2. Accessed July 14, 2022.

54. Mombelli A. Antimicrobial therapy as adjunct to periodontal treatment: Effect of timing GAR. https://trialsearch.who.int/Trial2.aspx?TrialID=NCT02197260. Accessed July 14, 2022.

55. Cosgarea R. Comparing 3 versus 7 days systemic administration of Amoxicillin (AMX) and Metronidazole (MET) in severe chronic periodontitis patients. https://trialsearch.who.int/Trial2.aspx?TrialID=ISRCTN17605083. Accessed July 14, 2022.

56. Owen M. Adjunctive systemic azithromycin versus amoxicillin and metronidazole in non-surgical mechanical therapy of periodontitis. https://trialsearch.who.int/Trial2.aspx?TrialID=ACTRN12615001047583. Accessed July 14, 2022.

57. Feres M. Dose and duration of metronidazole and amoxicillin for treatment of chronic periodontitis. https://trialsearch.who.int/Trial2.aspx?TrialID=NCT02735395. Accessed July 14, 2022.

58. Retamal-Valdes B. Amoxicillin and metronidazole during periodontal treatment. https://trialsearch.who.int/Trial2.aspx?TrialID=NCT02954393. Accessed July 14, 2022.

59. International Clinical Trials Registry Platform. The effects of periodontal therapy on glycemic control in diabetic patients. https://trialsearch.who.int/Trial2.aspx?TrialID=NCT03783845. Accessed July 14, 2022.

60. Lu H, Pengcheng H. Effect of SRP with concomitant administration of systemic amoxicillin and metronidazole on hematological parameters and lipid metabolism in patients with chronic periodontitis and type II diabetes mellitus. https://trialsearch.who.int/Trial2.aspx?TrialID=ChiCTR1900027377. Accessed July 14, 2022.

61. Theodoro LH. *Lactobacilli* associated with antibiotics in the treatment of periodontitis in smokers. https://trialsearch.who.int/Trial2.aspx?TrialID=RBR-55bc85. Accessed July 14, 2022.

62. Yan X. A randomized controlled clinical trial to evaluate the efficacy and safety of azithromycin in periodontal bone defect repair. https://trialsearch.who.int/Trial2.aspx?TrialID=ChiCTR2000035453. Accessed July 14, 2022.

63. Anuroopa P, Gulafsha M. Checking clinical effects of scaling and root planing with antibiotics and lasers in treatment of gum disease. https://trialsearch.who.int/Trial2.aspx?TrialID=CTRI/2020/11/029010. Accessed July 14, 2022.

64. Yaghini J. The effect of antibiotic therapy on oral health-related quality of life in patients. https://trialsearch.who.int/Trial2.aspx?TrialID=IRCT20201221049786N1. Accessed July 14, 2022.

65. Wu X. The mechanism, incidence rate, characteristics of malocclusion and orthodontic treatment effects of impacted tooth, ectopic tooth or/and periodontal disease or/and maxillary and mandibular discrepancy and so on and bracket bonding of aerosol reduction method during COVID-19 pandemic. https://trialsearch.who.int/Trial2.aspx?TrialID=ChiCTR2200056611. Accessed July 14, 2022.

66. ClinicalTrials.gov. Clinical and radiographic evaluation of NIPSA versus M-MIST in treatment of intra-osseous defects. NCT04149834. https://www.clinicaltrials.gov/study/NCT04149834. Accessed July 14, 2022.

67. ClinicalTrials.gov. Mechanisms and treatment response of aggressive periodontitis in children. NCT01330719. https://www.clinicaltrials.gov/study/NCT01330719. Accessed July 14, 2022.

68. ClinicalTrials.gov. Effect of antibiotics on systemic inflammation. NCT05115201. https://www.clinicaltrials.gov/study/NCT05115201. Accessed July 14, 2022.

69. ClinicalTrials.gov. Surgical treatment of peri-implantitis with and without systemically adjunctive antibiotics. NCT02185209. https://www.clinicaltrials.gov/study/NCT02185209. Accessed July 14, 2022.

70. ClinicalTrials.gov. Impact of non-surgical periodontal therapy on oral and gut microbiome. NCT04580355. https://www.clinicaltrials.gov/study/NCT04580355. Accessed July 14, 2022.

71. ClinicalTrials.gov. Antibiotics as adjuncts to periodontal therapy: Pharmacokinetic considerations and dosing strategies. NCT04669717. https://www.clinicaltrials.gov/study/NCT04669717. Accessed July 14, 2022.

72. Santi SS, Gomes M, Ziegelmann P, Zanatta FB. What combination protocol of non-surgical periodontal therapy plus systemic amoxicillin/metronidazole (AMX/MET) will provide the best clinical outcomes in the treatment of aggressive periodontitis? A meta-analysis. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=55697. Accessed July 14, 2022.

73. Liaw A, Nimmo A, Da Silva B. The role of systemic antibiotic adjuncts in expediting the periodontal tissue healing process following non-surgical scaling and root planing in patients with chronic periodontitis: A systematic review. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=43571. Accessed July 14, 2022.

74. Botelho J, Machado V. Systemic antibiotics in the treatment of periodontitis: A systematic review and meta-analysis of randomized clinical trials. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=127494. Accessed July 14, 2022.

75. Lim SJ, Ivanovski S, McGowen K. Is there a benefit in adjunctive azithromycin in non-surgical therapy for periodontitis: A systemic review of randomised controlled trials. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=71702. Accessed July 14, 2022.

76. Baghani Z, Shabestari SB, Karrabi M. Extent of clinical attachment loss as a practical parameter in efficacy of adjunctive photodynamic therapy in stage II–IV, grade C molar-incisor pattern periodontitis (aggressive periodontitis): A systematic review and meta-analysis. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=321211. Accessed July 14, 2022.

77. Hewakosgoda M, Alles N, Soysa N. Efficacy of systemically administered metronidazole with or without amoxicillin as an adjunct in the treatment of periodontitis in smoker and non-smoker adults. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=270475. Accessed July 14, 2022.

78. Chambrone L, Vargas del Campo M, Silie Arboleda S, Serna MJ, Guerrero MP, de Souza JG. Efficacy of adjunctive antibiotic therapy in scaling and root planing treatment of smokers with chronic periodontitis: A systematic review. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=19061. Accessed July 14, 2022.

79. Zhang Z, Chen F, Li W, Wang W. Comparing the periodontitis recovery of adjunctive amoxicillin/metronidazole and azithromycin: A systematic review and meta-analysis. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=331780. Accessed July 14, 2022.

80. Jones O, Hoyle P. Azithromycin in the treatment of rapidly progressing periodontal disease: A systematic review. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=168195. Accessed July 14, 2022.

81. Chopra A, Banerjee A, Puzhankara L, Venkitachalam R, Kedlaya M. Are probiotics an effective alternative to conventional antimicrobials agents for the management of periodontal diseases: A systematic review and meta-analysis. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=209253. Accessed July 14, 2022.

82. McGowan K, McGowan T, Ivanovski S. Amoxicillin and metronidazole prescribed in combination as an adjunct to non-surgical periodontal therapy: A systematic review of randomised-controlled trials. https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=64632. Accessed July 14, 2022.

83. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Ann Periodontol.* 1999;4(1):1–6. doi:10.1902/annals.1999.4.1.1

84. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. *J Periodontol.* 2018;89(S1):S1–S8. doi:10.1002/JPER.18-0157

Clinical and radiographic success rate of the root canal filling materials used in primary teeth: A systematic review

Lavanya Govindaraju[1,A,B,D], Ganesh Jeevanandan[1,A,B,D], Satish Vishwanathaiah[2,A,C,E,F], Prabhadevi Chandrashekara Gowda Maganur[2,B,E]

[1] Department of Pedodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Science, Saveetha University, Chennai, India

[2] Division of Pedodontics, Department of Preventive Dental Sciences, College of Dentistry, Jazan University, Saudi Arabia

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

Address for correspondence
Prabhadevi Chandrashekara Gowda Maganur
E-mail: Prabhadevi.maganur@gmail.com

Abstract

One of the most important factors that determine the success of pulpectomy in primary teeth is the root canal filling material used. This systematic review is an update on the success rates of various materials used for obturation in primary teeth. An electronic search was carried out in the PubMed, Scopus, Web of Science, and Cochrane Library databases with the preset inclusion and exclusion criteria. Only randomized or quasi-randomized clinical and controlled trials with a minimum follow-up of 12 months were included for analysis. Nine articles were considered potentially eligible for inclusion in this review. All the included trials had zinc oxide−eugenol (ZOE) cement as a control group. The time span of the included trials extended from 12 to 30 months. Only 2 trials were at low risk of bias. Evidence to support the success rates of obturating materials used in primary teeth is scarce, which necessitates further high-quality randomized controlled clinical trials with regard to this issue.

Keywords: systematic review, deciduous tooth, obturating materials, primary tooth, meta-analysis

Introduction

Dental caries in primary teeth is the most pervasive chronic disease, with a prevalence of approx. 60–90% among children. If untreated, this disease may cause irreversible pulpitis requiring root canal treatment. This, in turn, affects the general health of children and disrupts their quality of life.[1–3] The management of dental caries in primary teeth is extremely challenging for pediatric dentists, and endodontic treatment is the only option to treat and preserve primary teeth with chronic irreversible pulpitis in a symptomless state until they are replaced by permanent teeth. This treatment is achieved via a procedure called pulpectomy, which includes the chemo-mechanical preparation of the root canals of primary teeth. The prepared root canals are filled with a material that should have maximal antibacterial effects and the ability to resorb at the same rate as primary teeth.[4] The complexity of the root canal morphology, along with the process of physiological resorption in primary teeth, is an inherent hindrance to effective instrumentation, and therefore, an ideal obturating material is needed to ascertain the success of root canal treatment.[5,6]

There are abundant obturating materials for primary teeth available on the market, but none of these materials meets the requirements completely.[7] Until 2008, zinc oxide–eugenol (ZOE) cement was the only root canal filling material for endodontically treated primary teeth.[8] However, ZOE cement has limited antimicrobial action and a slow resorption rate, and if it is extruded beyond the apex, it can cause irritation to the periapical tissues, lead to the necrosis of the bone and cementum, and reorient the path of eruption among permanent teeth.[9–11]

Therefore, it was necessary to find an appropriate replacement for ZOE cement among root canal filling materials for primary teeth, one of them being iodoform paste.[12] Iodoform was introduced for obturation in primary teeth; despite having good clinical and radiographic success rates, it accelerates the root resorption of primary teeth.[13] Additionally, it produces yellowish discoloration of the tooth, which, in turn, has a negative esthetic effect.[14] The major drawback of using calcium hydroxide $(Ca(OH)_2)$ as an obturating material is that it provokes inflammatory root resorption in primary teeth when in proximity to any vital pulp tissue.[15] Currently, the combination of $Ca(OH)_2$ and iodoform paste is used in practice, given its potent antimicrobial properties and an easy resorption rate when extruded without any foreign body reactions. However, the material resorbs faster than the tooth, resulting in a hollow tube effect that leads to uncertainty regarding the prognosis of the pulpectomized tooth.[10,16] Recently, Endoflas was introduced, comprising ZOE cement, $Ca(OH)_2$ and iodoform; this material was proposed to have all the desirable properties of an ideal obturating material in primary teeth.[17] As none of the abovementioned materials can be considered the optimal obturating material in deciduous teeth, the search for better materials continues via the modification of the existing materials with new components.[7,18–22]

Owing to the complex, varied morphology of primary root canals, the complete removal of the pulp tissue is not always possible, and therefore, the obturating material used largely contributes to the prognosis and success of pulpectomy-treated primary teeth. Although there are numerous options available for one to choose as the close-to-ideal root canal filling material in primary teeth, there is no consensus among pediatric practitioners regarding the best available material for obturation in primary teeth. Hence, the present systematic review aimed to critically assess the available dental literature on the clinical and radiographic success rates of various obturating materials used for pulpectomy in primary teeth.

Material and methods

Search methodology for trial identification

The systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42021286974), and was conducted in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 statement.[23] The PubMed, Scopus, Web of Science, and Cochrane Library electronic databases were searched without any date and language restrictions. The preset inclusion and exclusion criteria were used during the search. The authors manually searched for additional eligible trials. Other review articles were also examined to retrieve relevant references. The search terms used were: 'primary teeth'; 'deciduous tooth'; 'deciduous dentition'; 'primary dentition'; 'children'; 'obturating materials'; 'zinc oxide eugenol'; 'calcium hydroxide'; 'iodoform'; 'endoflas'; 'root canal filling material'; 'clinical success rate'; 'radiographic success rate'; and 'quality of obturation'.

Criteria for the inclusion of trials

Only randomized or quasi-randomized clinical and controlled trials with a minimum follow-up of 12 months were included for analysis. Trials with less than 1 year of follow-up were excluded. Additionally, observational studies and pilot studies were excluded from this review.

Included participants: children between 4 and 9 years of age.

Intervention: any form of obturating material for primary teeth, including both conventional and newly experimented materials.

Type of outcome measures: the primary outcome measure in this review was the clinical and radiographic success rates; the quality of obturation was considered a secondary outcome.

Collection and analysis of data

The screening for eligibility for the trials was performed independently by 2 review authors. Duplicate studies and studies that did not meet the inclusion criteria were excluded. Data regarding the general characteristics and outcome variables of the included studies were extracted. The criteria used for assessing the clinical and radiological success rates were the same for each study. The quality of the included studies was assessed using the Consolidated Standards of Reporting Trials (CONSORT) guidelines.[24]

Assessment of the risk of bias of the included trials

The risk of bias was appraised by 2 authors independently, and any difference in opinion between them was sorted out by discussion or by involving another pediatric dentist. The assessment of the risk of bias was carried out using a refined and adapted tool of the Cochrane Collaboration. Five domains were assessed, namely, sample size determination, random sequence generation, allocation concealment, blinding of the participants/personnel, and blinding of the outcome assessment. Each domain was recorded as either low or high risk based on the information reported in the study. A judgment of 'unclear risk of bias' was marked when the trial methods were unclear and until any further information from the authors involved in the trial was available. The overall risk of bias in the included trials was then assessed as follows[25]:

– low risk of bias – when all 5 domains were assessed as low risk of bias;
– high risk of bias – when at least one domain was assessed as high risk of bias; and
– unclear risk of bias – when at least one domain was assessed as unclear risk of bias, but none of the domains were assessed as high risk of bias.

Results

After thoroughly searching the electronic databases and other sources, 146 articles were identified. Of these, 129 articles were excluded after reading the title, as the records were irrelevant. After removing duplicates, 12 articles were screened, and 3 articles were further excluded, as one of them was not a randomized controlled trial, and the other 2 had a follow-up period shorter than 12 months.[22,26,27] Nine articles were considered potentially eligible for this review.[7,10,17,28–33] The Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) flowchart is shown in Fig. 1.

Characteristics of the included studies

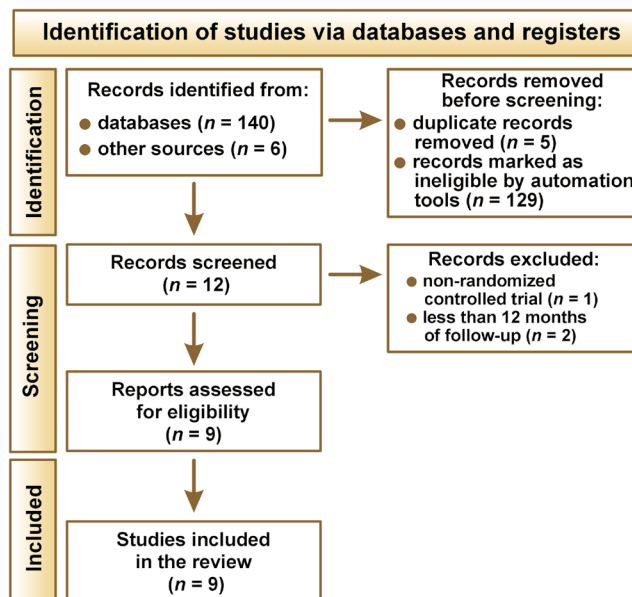We included 9 trials in this review, all of which were published between 2004 and 2020. One trial was self-funded,[30]

Fig. 1. Flowchart of the study according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines

1 trial received a grant from the Program for New Clinical Techniques and Therapies of Peking University School and Hospital of Stomatology, China,[7] 2 trials mentioned that they did not receive any financial support,[32,33] and the remaining 5 studies provided no information relating to the funding sources.[10,17,28,29,31] The characteristics of the included studies are depicted in Table 1.

Design and methods

Four studies were randomized clinical trials (RCTs),[10,28,30,31] 2 studies were quasi-randomized trials,[17,29] and the other 3 studies were randomized controlled trials.[7,32,33] All the included trials used ZOE cement in one group, and 4 studies had multiple-treatment groups that were compared with ZOE cement.[7,17,30,31] The study duration of the included trials (the follow-up period) ranged from 12 to 30 months.

Participants

The trials included participants aged 4–9 years, with a total of 726 teeth treated with pulpectomy.

Interventions

All the included trials used ZOE cement in one group. Vitapex was tested in 4 trials,[7,10,28,30] Metapex in 1,[17] RC Fill in 1,[30] Endoflas was included as one of the test groups in 3 trials,[17,31,32] mixed primary root canal filling (MPRCF) was tested in 1 trial,[7] zinc oxide (ZnO)–ozonated sesame oil was tested in 1 trial,[29] another tested ZnO with the *Aloe vera* extract and ZnO with 10% sodium fluoride (NaF),[31] and the other trial the ZnO–propolis mixture.[33]

Outcome measures

All the trials reported the clinical and radiographic success rates of the obturating materials tested. Two trials also reported on the quality of obturation.[10,17]

Risk of bias of the included trials

Table 2 depicts the summary of the risk of bias assessment for the 9 trials included in the review. Two trials were found to have a low risk of bias,[7,33] and the remaining 7 studies were considered to have a high risk of bias.[10,17,28–32] Six included trials showed selection bias, as sample size determination or random sequence generation or allocation concealment were not achieved.[10,17,28,29,31,32] We considered 6 studies to have a high risk of performance bias, as the blinding of the participants was either not completed or not mentioned.[10,17,28–31] Five trials had a high risk of detection bias, as the outcome assessor was not blinded.[10,17,28,29,31]

Table 1. Characteristics of the included trials

Study	Methods	Participants' age [years]	Intervention	Primary outcome	Secondary outcome	Conclusion	Funding
Chen et al.[7] 2017	study design: double-blinded randomized controlled trial study duration: 18 months sample size: 160 teeth	5.88 ±1.27	group 1: ZOE group 2: Vitapex group 3: MPRCF	clinical success rate: at 6 months: ZOE – 100% Vitapex – 100% MPRCF – 100% at 12 months: ZOE – 100% Vitapex – 80.4% MPRCF – 100% at 18 months: ZOE – 92.2% Vitapex – 71.4% MPRCF – 96.2% statistically significant differences between ZOE and Vitapex, and Vitapex and MPRCF at 12 and 18 months ($p = 0.010$*) radiographic success rate: at 6 months: ZOE – 100% Vitapex – 94.5% MPRCF – 100% at 12 months: ZOE – 100% Vitapex – 60.7% MPRCF – 100% at 18 months: ZOE – 88.2% Vitapex – 53.6% MPRCF – 92.5% statistically significant differences between ZOE and Vitapex, and Vitapex and MPRCF at 12 and 18 months ($p = 0.010$*)	–	MPRCF shows better clinical and radiographic success rates than Vitapex, and similar success rates with ZOE	received a grant from the Program for New Clinical Techniques and Therapies of Peking University School and Hospital of Stomatology, China
Mortazavi and Mesbahi[10] 2004	study design: RCT study duration: 10–16 months sample size: 58 teeth	5.8 ±1.9 (ZOE group) 5.6 ±1.1 (Vitapex group)	group 1: ZOE group 2: Vitapex	clinical success rate: ZOE – 78.9% Vitapex – 100% radiographic success rate: ZOE – 75.0% Vitapex – 100% $p = 0.015$*	quality of obturation: under fill: ZOE – 31.2% Vitapex –11.5% optimal fill: ZOE – 46.8% Vitapex – 50.0% over fill: ZOE – 21.8% Vitapex – 38.4%	Vitapex is significantly more successful than ZOE for pulpectomy in necrotic primary teeth	not mentioned

Study	Methods	Participants' age [years]	Intervention	Primary outcome	Secondary outcome	Conclusion	Funding
Subramaniam et al.[17] 2011	study design: quasi-RCT study duration: 18 months sample size: 45 teeth	5–9	group 1: Endoflas group 2: Metapex group 3: ZOE	clinical success rate: at 3, 6, 12, and 18 months: Endoflas – 93.3% Metapex – 100% ZOE – 93.3% radiographic success rate: at 3, 6, 12, and 18 months: Endoflas – 93.3% Metapex – 100% ZOE – 93.3% $p = 0.097$	quality of obturation: under fill: Endoflas – 20.0% Metapex – 13.3% ZOE – 20.0% optimal fill: Endoflas – 66.7% Metapex – 66.7% ZOE – 66.7% over fill: Endoflas – 13.3% Metapex – 20.0% ZOE – 13.3.% presence of voids: Endoflas – 13.3% Metapex – 20.0% ZOE – 13.3.% $p = 1.000$	Metapex showed a higher success rate as a root canal filling material; however, there was no significant difference between the 3 root canal filling materials	not mentioned
Trairatvorakul et al.[28] 2008	study design: RCT study duration: 12 months sample size: 54 teeth	5.6 ±1.2	group 1: ZOE group 2: Vitapex	clinical success rate: at 6 months: ZOE – 96% Vitapex – 100% at 12 months: ZOE – 93% Vitapex – 96% $p = 1.000$ radiographic success rate: at 12 month:s ZOE – 85% Vitapex – 89% $p = 1.000$	–	ZOE and Vitapex yielded similar success	not mentioned
Chandra et al.[29] 2014	study design: quasi-randomized controlled trial study duration: 12 months sample size: 60 teeth	3.8–7.6	control group: ZOE test group: ZnO plus ozonated sesame oil	clinical success rate: at 12 months: ZnO + ozonated sesame oil – 100% ZOE – 96.7% radiographic success rate: at 12 months: ZnO + ozonated sesame oil – 93.3% ZOE – 70.0% $p = 0.408$	–	ZnO + ozonated sesame oil can be considered as an alternative obturating material	not mentioned
Pramila et al.[30] 2016	study design: RCT study duration: 30 months sample size: 129 teeth	4–9 years	group 1: RC Fill group 2: Vitapex group 3: ZOE (Pulpdent Root Canal Sealer)	clinical success rate: at 6, 12, and 30 months: RC Fill – 100% Vitapex – 100% Pulpdent – 100% radiographic success rate: at 6 months: RC Fill – 89% Vitapex – 80% Pulpdent – 97% at 12 months: RC Fill – 94% Vitapex – 82% Pulpdent – 97% at 30 months: RC Fill – 94% Vitapex – 90% Pulpdent – 97% $p > 0.05$	–	RC Fill, Vitapex and Pulpdent are equally effective root canal filling materials	self-funded

Study	Methods	Participants' age [years]	Intervention	Primary outcome	Secondary outcome	Conclusion	Funding
Goel et al.[31] 2018	study design: RCT study duration: 12 months sample size: 120 teeth	4–9	group 1: ZOE group 2: ZnO with *Aloe vera* extract group 3: ZnO with 10% NaF group 4: Endoflas	clinical success rate: at 3 months: ZOE – 96.7% ZnO + *Aloe vera* – 100% ZnO + NaF – 100% Endoflas – 100% at 6 months: ZOE – 89.7% ZnO + *Aloe vera* – 92.6% ZnO + NaF – 100% Endoflas – 96.6% at 9 months: ZOE – 82.8% ZnO + *Aloe vera* – 88.9% ZnO + NaF – 96.4% Endoflas – 96.3% at 12 months: ZOE – 74.1% ZnO + *Aloe vera* – 83.3% ZnO + NaF – 92.9% Endoflas – 96.3% radiographic success rate: at 3 months: ZOE – 96.7% ZnO + *Aloe vera* – 100% ZnO + NaF – 96.6% Endoflas – 100% at 6 months: ZOE – 82.8% ZnO + *Aloe vera* – 88.9% ZnO + NaF – 96.6% Endoflas – 96.6% at 9 months: ZOE – 72.4% ZnO + *Aloe vera* – 85.2% ZnO + NaF – 89.3% Endoflas – 96.3% at 12 months: ZOE – 63.0% ZnO + *Aloe vera* – 79.2% ZnO + NaF – 85.7% Endoflas – 88.9% $p > 0.05$	–	Endoflas shows the highest success rate, followed by ZnO + NaF and ZnO + *Aloe vera*; however, there was no significant difference between the 4 groups	not mentioned
Pandranki et al.[32] 2018	study design: randomized controlled trial study duration: 24 months sample size: 60 teeth	4–9	control group: ZOE test group: Endoflas	clinical success rate: at 12 months: Endoflas – 92% ZOE – 89% at 24 months: Endoflas – 68% ZOE – 74% $p = 0.629$ radiographic success rate: at 24 months: Endoflas – 56% ZOE – 52% $p = 0.797$	–	Endoflas is a potential alternative to ZOE for obturation in primary teeth	none

Study	Methods	Participants' age [years]	Intervention	Primary outcome	Secondary outcome	Conclusion	Funding
RojaRamya et al.[33] 2020	study design: 2-arm, parallel-group randomized controlled trial

study duration: 24 months

sample size: 40 teeth | 4–8 | control group: ZOE

test group: ZnO–propolis mixture | overall success rate:

at 6 months: ZnO–propolis – 100% ZOE – 80%

$p = 0.035$*

at 12 months: ZnO–propolis – 95% ZOE – 80%

$p = 0.151$

at 24 months: ZnO–propolis – 95% ZOE – 70%

$p = 0.037$* | – | the ZnO–propolis mixture is a better alternative to ZOE | none |

RCT – randomized clinical trial; ZOE – zinc oxide–eugenol; ZnO – zinc oxide; MPRCF – mixed primary root canal filling; NaF – sodium fluoride; * statistically significant.

Table 2. Risk of bias of the included trials

Study	Sample size determination	Random sequence generation	Allocation concealment	Blinding of the participants	Blinding of the outcome assessment	Risk of bias
Chen et al.[7] 2017	yes	yes	yes	yes	yes	low
Mortazavi and Mesbahi[10] 2004	no	yes	no	no	no	high
Subramaniam et al.[17] 2011	no	no	no	no	no	high
Trairatvorakul et al.[28] 2008	no	yes	no	no	no	high
Chandra et al.[29] 2014	no	no	no	no	no	high
Pramila et al.[30] 2016	yes	yes	yes	no	yes	high
Goel et al.[31] 2018	yes	yes	no	no	no	high
Pandranki et al.[32] 2018	no	yes	no	yes	yes	high
RojaRamya et al.[33] 2020	yes	yes	yes	yes	yes	low

Discussion

This systematic review was intended to evaluate the clinical and radiographic success rates of various obturating materials used in primary teeth. Eleven different obturating materials were used in the 9 trials included for review. For all of the trials included, ZOE cement was used as one of the obturating materials or control groups. Other materials evaluated included Vitapex, Metapex and Endoflas, all of which are conventionally used materials. New materials included ZnO with iodoform, MPRCF, ZnO–ozonated sesame oil, ZnO with the *Aloe vera* extract, ZnO with 10% NaF, and the ZnO–propolis mixture.

The secondary intent of this review was to determine the quality of obturation with these root canal materials in primary teeth. There were only 2 trials that evaluated the quality of obturation[10,17]; however, statistical significance was not mentioned to detect a clear relationship between the obturating material and the quality of obturation. This

indicates that there are no studies in the literature determining the relationship between the obturating material used and the quality of obturation.

In terms of methodological limitations of the included trials, only 2 trials were found to have a low risk of bias,[7,33] whereas others were found to have a high risk of bias. The domain most commonly found to be at high risk of bias was allocation concealment and blinding of the participants, followed by blinding of the outcome assessor and sample size determination.

This review found, in the head-to-head comparisons, that each of the examined obturating materials was more effective than ZOE cement. However, most of the included trials had a high risk of bias, and hence, we could not draw any definite conclusions. Taking studies with a low risk of bias into consideration, MPRCF and the ZnO–propolis mixture were better alternatives to ZOE cement.

The MPRCF contains 0.28 g of ZnO, 0.18 g of iodoform, and 0.01 g of $Ca(OH)_2$. Notably, the composition of MPRCF

is similar to that of Endoflas, which is considered close to the ideal root canal filling material in primary teeth. The trials that tested Endoflas also showed a high success rate despite having a high risk of bias.[17,31,32] Hence, more research should be focused on developing an ideal root canal filling material incorporating these components.

The other alternative is the ZnO–propolis mixture that replaces eugenol with propolis. Propolis is proven to have antimicrobial and anti-inflammatory properties.[34,35] However, the preparation of the mixture and the concentrations of the ingredients were not mentioned in the included trial.[33] Another important fact that should not be neglected is that any new alternative to ZOE cement, including both MPRCF and the ZnO–propolis mixture, still contains ZnO as one of its key components. Studies have shown that ZOE cement shows good resistance to bacterial leakage and inhibits most organisms,[36,37] indicating that ZnO is an inevitable component of any new alternative.

Unfortunately, the trials included in this review were all heterogeneous, and hence, we were not able to perform a meta-analysis. Evidence from the trials included in this review cannot be applied to clinical practice due to a major void in the methodological constraints. With pulpectomy being the major procedure in pediatric dental practice, it is surprising to know that no qualitative studies are assessing the quality of obturation and the success rates of the obturating materials used in primary teeth. Future trials must be well-designed RCTs in accordance with the CONSORT statement, and all possible measures should be taken to prevent any sort of bias.

Conclusions

Based on the scientific information available, there seems to be a low level of evidence to support the success rates of a new ideal root canal filling material for primary teeth. High-quality randomized controlled clinical trials with a minimum of 12 months of follow-up are needed to decide upon the best obturating material for primary teeth, as at present, there is no consensus among pediatric dentists with regard to this issue.

Ethics approval and consent to participate

Not applicable.

Data availability

All data generated and/or analyzed during this study is included in this published article.

Consent for publication

Not applicable.

ORCID iDs

Lavanya Govindaraju https://orcid.org/0000-0003-0249-5513
Ganesh Jeevanandan https://orcid.org/0000-0003-3631-6982
Satish Vishwanathaiah https://orcid.org/0000-0002-8376-297X
Prabhadevi Chandrashekara Gowda Maganur
https://orcid.org/0000-0002-0959-2597

References

1. Marcenes W, Kassebaum NJ, Bernabé E, et al. Global burden of oral conditions in 1990–2010: A systematic analysis. *J Dent Res*. 2013;92(7):592–597. doi:10.1177/0022034513490168
2. Petersson LG, Svanholm I, Andersson H, Magnusson K. Approximal caries development following intensive fluoride mouthrinsing in teenagers. A 3-year radiographic study. *Eur J Oral Sci*. 1998;106(6):1048–1051. PMID:9879918.
3. Sheiham A. Dietary effects on dental diseases. *Public Health Nutr*. 2001;4(2B):569–591. doi:10.1079/phn2001142
4. Machida Y. Root canal obturation in deciduous teeth [in Japanese]. *Nihon Shika Ishikai Zasshi*. 1983;36(7):796–802. PMID:6589308.
5. Kopel HM. Root canal therapy for primary teeth. *J Mich State Dent Assoc*. 1970;52(2):28–33. PMID:5263926.
6. Rifkin A. A simple, effective, safe technique for the root canal treatment of abscessed primary teeth. *ASDC J Dent Child*. 1980;47(6):435–441. PMID:6934178.
7. Chen X, Liu X, Zhong J. Clinical and radiographic evaluation of pulpectomy in primary teeth: A 18-months clinical randomized controlled trial. *Head Face Med*. 2017;13(1):12. doi:10.1186/s13005-017-0145-1
8. American Academy of Pediatric Dentistry Clinical Affairs Committee – Pulp Therapy Subcommittee; American Academy of Pediatric Dentistry Council on Clinical Affairs. Guideline on pulp therapy for primary and young permanent teeth. *Pediatr Dent*. 2005;27(7 Suppl):130–134. PMID:16541910.
9. Coll JA, Sadrian R. Predicting pulpectomy success and its relationship to exfoliation and succedaneous dentition. *Pediatr Dent*. 1996;18(1):57–63. PMID:8668572.
10. Mortazavi M, Mesbahi M. Comparison of zinc oxide and eugenol, and Vitapex for root canal treatment of necrotic primary teeth. *Int J Paediatr Dent*. 2004;14(6):417–424. doi:10.1111/j.1365-263X.2004.00544.x
11. Ozalp N, Saroğlu I, Sönmez H. Evaluation of various root canal filling materials in primary molar pulpectomies: An in vivo study. *Am J Dent*. 2005;18(6):347–350. PMID:16433405.
12. American Academy on Pediatric Dentistry Clinical Affairs Committee – Pulp Therapy Subcommittee; American Academy on Pediatric Dentistry Council on Clinical Affairs. Guideline on pulp therapy for primary and young permanent teeth. *Pediatr Dent*. 2008;30(7 Suppl):170–174. PMID:19216417.
13. Moskovitz M, Tickotsky N, Ashkar H, Holan G. Degree of root resorption after root canal treatment with iodoform-containing filling material in primary molars. *Quintessence Int*. 2012;43(5):361–368. PMID:22536587.
14. Garcia-Godoy F. Evaluation of an iodoform paste in root canal therapy for infected primary teeth. *ASDC J Dent Child*. 1987;54(1):30–34. PMID:3468139.
15. Ravi GR. Subramanyam RV. Calcium hydroxide-induced resorption of deciduous teeth: A possible explanation. *Dent Hypotheses*. 2012;3(3):90–44. doi:10.4103/2155-8213.103910
16. Gupta S, Das G. Clinical and radiographic evaluation of zinc oxide eugenol and metapex in root canal treatment of primary teeth. *J Indian Soc Pedod Prev Dent*. 2011;29(3):222–228. doi:10.4103/0970-4388.85829
17. Subramaniam P, Gilhotra K. Endoflas, zinc oxide eugenol and metapex as root canal filling materials in primary molars – a comparative clinical study. *J Clin Pediatr Dent*. 2011;35(4):365–369. doi:10.17796/jcpd.35.4.1377v06621143233
18. Al-Ostwani AO, Al-Monaqel BM, Al-Tinawi MK. A clinical and radiographic study of four different root canal fillings in primary molars. *J Indian Soc Pedod Prev Dent*. 2016;34(1):55–59. doi:10.4103/0970-4388.175515
19. Nakornchai S, Banditsing P, Visetratana N. Clinical evaluation of 3Mix and Vitapex as treatment options for pulpally involved primary molars. *Int J Paediatr Dent*. 2010;20(3):214–221. doi:10.1111/j.1365-263X.2010.01044.x

20. Khairwa A, Bhat M, Sharma R, Satish V, Maganur P, Goyal AK. Clinical and radiographic evaluation of zinc oxide with aloe vera as an obturating material in pulpectomy: An in vivo study. *J Indian Soc Pedod Prev Dent*. 2014;32(1):33–38. doi:10.4103/0970-4388.127051

21. Agarwal S, Gupta S, Tandon S, et al. Comparative evaluation of *Ocimum sanctum* and calcium hydroxide mix as an obturating material in deciduous molars: An in vivo study. *Int J Clin Pediatr Dent*. 2020;13(6):617–621. doi:10.5005/jp-journals-10005-1840

22. Gupta B, Singh I, Goyal P, Garg S, Gupta S. A clinical and radiographic study of four different root canal filling materials in primary molars – an in vivo study. *Dent J Adv Stud*. 2019;7:61–65. doi:10.1055/s-0039-1692611

23. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *Syst Rev*. 2021;10(1)89. doi:10.1186/s13643-021-01626-4

24. Moher D, Schulz KF, Altman DG; CONSORT Group. The CONSORT statement: Revised recommendations for improving the quality of reports of parallel-group randomised trials. *Clin Oral Investig*. 2003;7(1):2–7. doi:10.1007/s00784-002-0188-x

25. Whiting P, Savović J, Higgins JP, et al. ROBIS: A new tool to assess risk of bias in systematic reviews was developed. *J Clin Epidemiol*. 2016;69:225–234. doi:10.1016/j.jclinepi.2015.06.005

26. Chawla HS, Setia S, Gupta N, Gauba K, Goyal A. Evaluation of a mixture of zinc oxide, calcium hydroxide, and sodium fluoride as a new root canal filling material for primary teeth. *J Indian Soc Pedod Prev Dent*. 2008;26(2):53–58. doi:10.4103/0970-4388.41616

27. Ramar K, Mungara J. Clinical and radiographic evaluation of pulpectomies using three root canal filling materials: An in-vivo study. *J Indian Soc Pedod Prev Dent*. 2010;28(1):25–29. doi:10.4103/0970-4388.60481

28. Trairatvorakul C, Chunlasikaiwan S. Success of pulpectomy with zinc oxide-eugenol vs calcium hydroxide/iodoform paste in primary molars: A clinical study. *Pediatr Dent*. 2008;30(4):303–308. PMID:18767509.

29. Chandra SP, Chandrasekhar R, Uloopi KS, Vinay C, Kumar NM. Success of root fillings with zinc oxide-ozonated oil in primary molars: Preliminary results. *Eur Arch Paediatr Dent*. 2014;15(3):191–195. doi:10.1007/s40368-013-0094-8

30. Pramila R, Muthu MS, Deepa G, Farzan JM, Rodrigues SJ. Pulpectomies in primary mandibular molars: A comparison of outcomes using three root filling materials. *Int Endod J*. 2016;49(5):413–421. doi:10.1111/iej.12478

31. Goel H, Mathur S, Sachdev V. Clinical and radiographic evaluation of four different zinc-oxide integrated root canal obturating materials used in primary teeth. *Paediatr Dent J*. 2018;28(2):73–86. doi:10.1016/j.pdj.2018.06.002

32. Pandranki J, Vanga NR, Chandrabhatla SK. Zinc oxide eugenol and Endoflas pulpectomy in primary molars: 24-month clinical and radiographic evaluation. *J Indian Soc Pedod Prev Dent*. 2018;36(2):173–180. doi:10.4103/JISPPD.JISPPD_1179_17

33. RojaRamya KS, Vinay C, Uloopi KS, Chandrasekhar R. In vivo evaluation of zinc oxide–propolis mixture as root canal filling material in the primary molars: A 24-month follow-up randomized controlled trial. *J Indian Soc Pedod Prev Dent*. 2020;38(2):171–176. doi:10.4103/JISPPD.JISPPD_127_20

34. De Andrade Ferreira FB, Torres SA, Da Silva Rosa OP, et al. Antimicrobial effect of propolis and other substances against selected endodontic pathogens. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007;104(5):709–716. doi:10.1016/j.tripleo.2007.05.019

35. Tan-No K, Nakajima T, Shoji T, et al. Anti-inflammatory effect of propolis through inhibition of nitric oxide production on carrageenin-induced mouse paw edema. *Biol Pharm Bull*. 2006;29(1):96–99. doi:10.1248/bpb.29.96

36. Sisodia R, Ravi KS, Shashikiran ND, Singla S, Kulkarni V. Bacterial penetration along different root canal fillings in the presence or absence of smear layer in primary teeth. *J Clin Pediatr Dent*. 2014;38(3):229–234. doi:10.17796/jcpd.38.3.q8w63111t2354786

37. Priya MH, Bhat SS, Hegde KS. Comparative evaluation of bactericidal potential of four root canal filling materials against microflora of infected non-vital primary teeth. *J Clin Pediatr Dent*. 2010;35(1):23–29. doi:10.17796/jcpd.35.1.u57p4500360g2752

Advanced restorative management of focal microdontia: A brief review and case report

Carlos Alberto Jurado[1,2,A,B,D,F], Jose Villalobos-Tinoco[3,B,C,F], Abdulrahman Alshabib[4,5,B,C,F], Kelvin Ian Afrashtehfar[6,7,A,C,E,F]

[1] Operative Dentistry Division, Department of General Dentistry, College of Dentistry, University of Tennessee Health Science Center, Memphis, USA

[2] Department of Prosthodontics, College of Dentistry and Dental Clinics, University of Iowa, Iowa City, USA

[3] Department of Periodontology, School of Dentistry, National University of Rosario, Argentina

[4] Department of Restorative Dentistry, College of Dentistry, King Saud University, Riyadh, Saudi Arabia

[5] Engineer Abdullah Bugshan Research Chair for Dental and Oral Rehabilitation, King Saud University, Riyadh, Saudi Arabia

[6] Department of Clinical Sciences, Evidence-Based Practice Unit, College of Dentistry, Ajman University, United Arab Emirates

[7] Department of Reconstructive Dentistry and Gerodontology, School of Dental Medicine, University of Bern, Switzerland

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

Address for correspondence
Kelvin Ian Afrashtehfar
E-mail: kelvin.afrashtehfar@unibe.ch

Abstract

Focal microdontia is a dental anomaly characterized by the presence of a single abnormally small anterior or posterior tooth. The objective of this article is to provide an updated review of the literature on the advanced restorative management of focal microdontia, and to document a clinical case where the reviewed advanced restorative approaches were applied to treat a young adult presenting with a non-syndromic asymmetrical focal microdontia. We conducted a preliminary examination of the existing literature on the advanced restorative management of focal microdontia. Additionally, we presented a minimally invasive approach to the treatment of an 18-year-old female patient with non-syndromic asymmetrical focal micro-dontia. The primary advantage of adhesive dentistry is that it can better preserve the structure of smaller teeth. A review of literature reveals a paucity of reports on localized microdontia in the maxillary anterior region of the mouth. However, novel minimally invasive restorative procedures satisfy patients' aesthetic and functional preferences. Well-executed additive diagnostic wax-ups and intraoral mock-ups can serve as a permanent restoration blueprint, providing predictable results for focal dental anomalies in the aesthetic zone. In conclusion, the use of minimally invasive dental approaches in young patients with focal microdontia can result in long-term satisfactory aesthetic outcomes.

Keywords: minimally invasive, microdontia, adhesive dentistry, aesthetic dentistry, dental anomaly

Introduction

Microdontia is a dental anomaly characterized by the presence of teeth smaller in size. It can cause malocclusion, aesthetic concerns and food trapping. Microdontia has been associated with genetic and environmental factors; however, its etiology remains unclear.[1] Genetic factors are involved in the complex etiology of microdontia and play a key role in its formation. Ectodermal, mesodermal and neural crest cells contribute to the development of the teeth, and the enamel organ determines the tooth size variation at the bell stage.[2] The crown formation is thought to be related to the oral epithelium, which is responsible for the initial shape of the crown.[3] Microdontia is the second most common dental abnormality after hypodontia. The condition occurs more frequently in females than males,[4,5] with a reported prevalence of 1.5–2% in the general population. Microdontia can be classified into 3 types: localized true or focal microdontia for a single tooth; relative generalized microdontia, in which teeth are of regular size but jaws are larger than normal; and true generalized microdontia, in which all teeth are smaller than normal but maxilla and mandible are of regular size (Table 1).[6] The etiology of microdontia is poorly understood. However, external factors such as radiotherapy, chemotherapy or pollutants are known to cause developmental dental problems.[7,8]

Material and methods

A straightforward MEDLINE (PubMed) search was conducted in December 2022 to identify relevant articles published between January 1970 and December 2022. The search terms used were "microdontia" AND ("diagnostics" OR "management" OR "treatment" OR "therapy") AND ("dental" OR "dentistry" OR "stomatology"). We considered reviews, systematic reviews, guidelines, statements, observational studies, clinical case reports, and case series. The articles included in this review were limited to those written in the English language. Letters, book chapters, case reports, and studies for which the full text was not available were excluded. Only publications addressing the protocol for diagnosing microdontia patients and potential or successful treatments were analyzed. Articles pertaining to extensive or invasive treatments in microdontia patients were excluded.

The study selection was conducted independently by 2 reviewers (CAJ and JVT), with any disagreements resolved by the third reviewer (KIA). No quantitative analysis (e.g., meta-analysis) was performed.

Diagnostics

The treatment management of true microdontia is dependent upon the number and location of teeth and the patient's aesthetic demands. There is a paucity of literature on localized microdontia in the maxillary anterior region of the mouth. This is heavily associated with patients' aesthetic concerns.[9] Effective communication between the patient, clinician and dental technician is vital to ascertain the patient's preferences and to inform them about the limitations of the proposed treatment.[10] If the treatment does not meet the patient's expectations, further options must be discussed to address as many aspects as possible.

Digital smile design

Diagnostic wax-up and digital smile design are essential diagnostic tools for providing tentative treatment in the aesthetic zone prior to any irreversible dental procedures.[11,12] A diagnostic wax-up allows for modifying the dimension of the teeth in the cast. Subsequently, a putty index can be fabricated to create an intraoral diagnostic mock-up.[13] Diagnostic mock-ups allow the patient to

Table 1. Summary of microdontia types, associated conditions and treatment options[4–9]

Type	Description	Associated conditions	Treatment
Focal microdontia	A single tooth is smaller than average. The maxilla and the mandible are of regular size. This condition typically affects maxillary teeth, with the lateral incisor being the tooth most likely to be affected.	• chemotherapy or radiation • cleft lip and cleft palate • pituitary dwarfism • Down syndrome • ectodermal dysplasia • Turner syndrome • Williams syndrome • oro-facial-digital syndrome • hypodontia • Hallermann–Streiff syndrome • Routhmund–Thomson syndrome	• ceramic veneers pros: conservative preparation; high aesthetic results; long-lasting restoration cons: high cost; many appointments needed; sensitivity to bonding procedures
Relative generalized microdontia	This condition affects all the dentition. The teeth are of regular size but the maxilla and the mandible are larger in size, which results in the teeth appearing smaller.		• direct composites pros: conservative preparation; cost-effective; fewer appointments needed; satisfactory outcomes cons: low fracture resistance; susceptibility to staining
True generalized microdontia	This condition affects all the dentition. The teeth are smaller than the average size. The maxilla and the mandible are of regular size.		• crowns pros: high fracture resistance; high aesthetic results; long-lasting restoration cons: high cost; many appointments needed; aggressive tooth preparation

have an intraoral prototype of the proposed restorations. The patient and clinician can modify the blueprint until the patient is satisfied, and keep a record of the changes so that the dental technician can replicate this information in the final prosthesis.[14,15]

If a patient presents with a smaller tooth within the arch, an additive wax-up can be employed to increase the length and width of the tooth without modifying its structure. This shape can then be impressed with a putty index guide for a conventional diagnostic mock-up.

Minimally invasive approach

A recent consensus statement has recommended the use of minimally invasive procedures for the treatment of patients with tooth malformations such as microdontia.[16] The objective of minimally invasive preparations is the preservation of most tooth and periodontal tissues. This concept has been promoted with ceramic veneers since the early 1980s.[17,18] The development of bonding procedures provides predictable long-term success. However, it is required to remove minimal tooth structure.[19] Maintaining the entire tooth preparation in enamel is vital for the long-term success of the procedure. A 12-year retrospective study concluded with a 99% survival rate for ceramic veneer preparations confined in enamel and 94% survival rate for veneers confined in enamel only at the margins.[20] These findings underscore the importance of maintaining minimally invasive tooth preparation in the enamel structure.

The amount of tooth structure removed during preparation is contingent upon the tooth's position within the arch. If a smaller tooth is rotated or protruded, a more aggressive tooth preparation is necessary to correct it in the final prosthesis. Thus, having an affected tooth in an optimal position eliminates the necessity of employing an aggressive preparation technique. After the initial evaluation, an additive wax-up and diagnostic mock-up are performed.

Management of intrinsic and extrinsic tooth discoloration

Teeth whitening has become a popular conservative measure for the management of tooth discoloration. A precise diagnosis of the etiology of tooth discoloration is essential for the appropriate management of patients who require whitening procedures.[21–23] Intrinsic or extrinsic factors can cause tooth discoloration. Intrinsic discoloration affects the interior layer of the teeth with deep stains or tooth defects caused by a variety of factors, including medication, systemic conditions and metabolic diseases. The treatment of intrinsic stains usually involves restorative procedures, such as veneers and crowns.

Extrinsic stains can be caused by wine, tobacco, products rich in polyphenols, inadequate oral hygiene, and surface changes or defects. Treatment for extrinsic stains may involve teeth whitening procedures.[24–26] These procedures have shown to be very successful, with patients reporting satisfaction with color changes after 2 weeks of bleaching gel application. The color demonstrated stability for up to 12 months.[27] Patients with microdontia in the visible smile area may have critical aesthetic concerns due to the discrepancy in size and space between teeth.

Oral hygiene is also a challenge for patients, as smaller teeth are often unable to make contact with the adjacent teeth, which can result in the accumulation of food debris on the interproximal surfaces.[28,29]

Laminate veneers

Dental laminate veneers can be fabricated from feldspathic porcelain for patients with high aesthetic demands who are seeking long-term success. Highly aesthetic results are required when treating 1 or 2 single teeth in the aesthetic zone. Many reports have demonstrated the efficacy of feldspathic porcelain in fulfilling aesthetic demands in anterior teeth.[30–34] A systematic review and meta-analysis evaluating porcelain veneers for anterior teeth concluded that the central incisor teeth had a success rate of 95% after 9 years of service.[35]

Clinical case

The objective of this case report is to illustrate the steps involved in the diagnostic wax-up, intraoral mock-up, minimally invasive tooth preparation, shade selection, and fabrication of a single hand-crafted ceramic veneer to successfully address the aesthetic concerns, oral hygiene difficulties and occlusal function in a young adult patient presenting with an individual tooth with microdontia in the aesthetic zone.

An 18-year-old Hispanic female presented to the clinic with the chief complaint of having a small tooth in the front and disliking the color of her teeth (Fig. 1). After clinical, photographic and radiographic evaluation, the patient was diagnosed with localized microdontia affecting the maxillary left central incisor, the space between central incisors, the left central incisor, and the left lateral incisor. The patient understood her clinical condition and claimed that no family member had a similar condition.

The patient acknowledged that a minimally invasive approach could be provided, involving teeth whitening for the anterior tooth and a single facial veneer restoration for the maxillary left central incisor. An in-office vital teeth whitening procedure was performed using 35% hydrogen peroxide (Philips Zoom WhiteSpeed; Philips, Amsterdam, the Netherlands) for 30 min, with a second
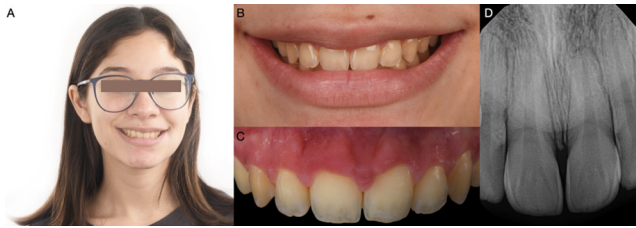
Fig. 1. Initial clinical situation

A. Extraoral frontal view; B. Extraoral close-up; C. Intraoral view;
D. Radiographic image.

session repeated 1 week later. An at-home bleaching product, containing 9% hydrogen peroxide, was recommended to the patient (Philips Sonicare Teeth Whitening Kit; Philips). The patient was instructed to apply the product daily for 2 weeks. After the in-office and at-home whitening treatments, the patient expressed satisfaction with the result (Fig. 2).

During the implementation of a minimally invasive approach, an additive diagnostic wax-up (GEO Classic; Renfert GmbH, Hilzingen, Germany) for the maxillary left central incisor was performed, matching the adjacent central incisor and closing the spaces (Fig. 3). A putty guide with light body impression material (elite P&P; Zhermack, Badia Polesine, Italy) and tooth reduction guides were made based on the diagnostic wax-up, and an intraoral mock-up was provided (Fig. 4). The diagnostic mock-up was placed in the patient's mouth, and the patient expressed satisfaction with the contours of the proposed restoration. Consequently, she requested to proceed with the restorative procedure (Fig. 5). Once the aesthetic approval and informed consent had been obtained, the tooth reduction was carried out based on the diagnostic mock-up. In order to maintain the conservative approach, the preparation was performed with the aid of reduction guides, and the final preparation was polished and smoothed with polishing discs (Sof-Lex™ discs; 3M, St. Paul, USA) (Fig. 6).

After evaluating the final preparation, the double cord impression technique was employed, with the first packing being 00 and the second packing being 0 (Ultrapak cord; Ultradent Products, Inc., South Jordan, USA), impregnated with a hemostatic agent without epinephrine

Fig. 3. Additive diagnostic wax-up

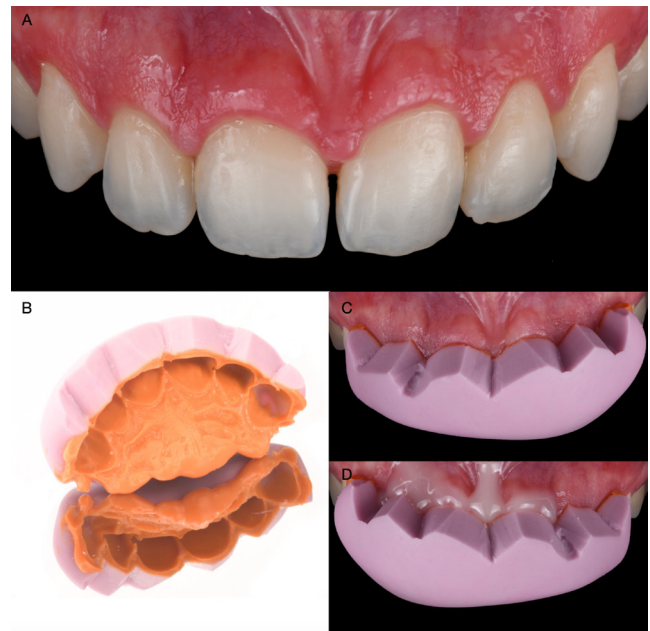A. Frontal view; B. Left view; C. Right view.

Fig. 4. Wax-up-based putty matrix for intraoral mock-up fabrication

A. Lubrication of the enamel surface; B. Putty index washed with light body material; C. Intraoral try-in of the putty guide; D. Guide insertion containing bis-acryl material for mock-up fabrication.
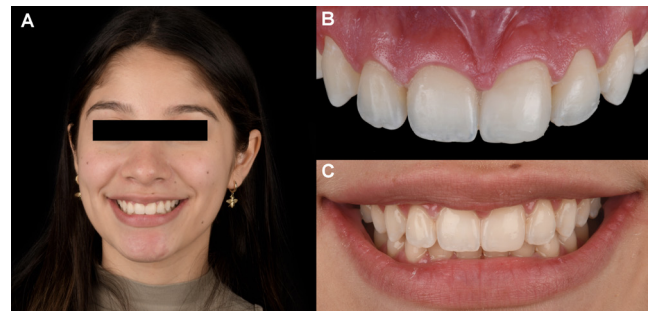

Fig. 2. Before (A) and after (B) teeth whitening procedure

Fig. 5. Assessment of the diagnostic mock-up

A. Extraoral frontal view of the dentofacial restorative proposal;
B. Intraoral frontal view of the dentogingival aesthetic proposal;
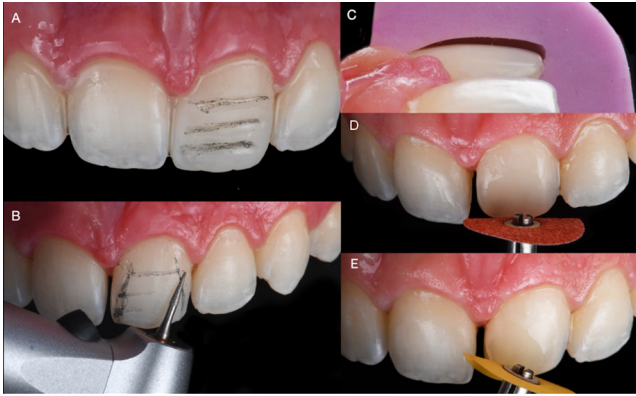C. Maximum intercuspation.

Fig. 6. Conservative tooth preparation

A. Horizontal reduction grooves; B. Line angles for tooth preparation; C. Evaluation with the use of reduction guides; D. Polishing with a coarse disc; E. Polishing with a fine disc.
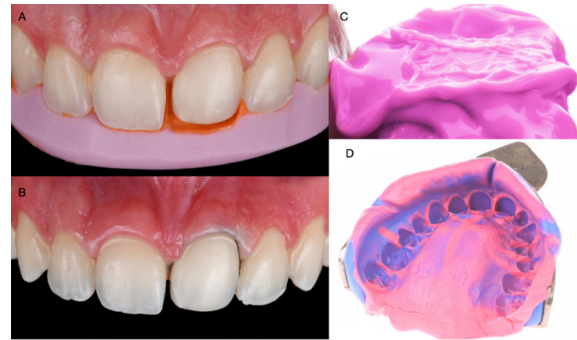

Fig. 7. Preparation of the final impression

A. Evaluation of the final preparation; B. Cord packing; C. Light and heavy body application; D. Final impression.

(Hemodent®; Premier Dental, Plymouth Meeting, USA). The final impression was prepared using light and heavy body viscosity polyvinyl siloxane (Virtual® 380; Ivoclar Vivadent Inc., Amherst, USA) (Fig. 7).

Intraoral photographs (EOS 1300D Canon, Canon EF 100 mm f/2.8L Macro IS; Canon Inc., Tokyo, Japan) were captured with a cross-polarization filter (polar_eyes; PhotoMed International, Van Nuys, USA) to evaluate the shade and substrate present. A light source was positioned on the palatal side to take another photograph that would appear orange in color. This photograph was used to evaluate the translucency of the enamel, with the goal of obtaining a value measurement (Fig. 8). Subsequently, all photographs and the final impression were sent to the dental technician. The final impression was poured out in type IV stone (GC FUJIROCK® EP; GC America, Alsip, USA). Feldspathic porcelain (Noritake Super Porcelain EX-3™; Kuraray, Yokyo, Japan) veneer was handcrafted based on the photographic information, and the photos of the patient's mouth and restoration were superimposed to evaluate the final shade (Fig. 9).

Tooth isolation was achieved with a rubber dam, which was secured from the maxillary left second premolar to the maxillary right second premolar with holding clamps (Rubber Dam #2; Hu-Friedy Manufacturing, Chicago, USA). An additional clamp (Hygenic Brinker Clamp B4; Coltène/Whaledent Inc., Cuyahoga Falls, USA) was placed on the tooth to ensure the optimal conditions for the bonding procedure. The adjacent teeth were isolated with polytetrafluoroethylene (PTFE) film (Masters® Orange T-Tape; GF Thompson Co., Ltd., Newmarket, Canada), and the maxillary left central incisor was initially treated with 32% phosphoric acid gel (Uni-Etch® w/BAC; BISCO Dental, Schaumburg, USA) for 30 s on the enamel surface, rinsed and gently dried. Then, the primer and adhesive were applied (OptiBond™ FL; Kerr Dental, Orange, USA) following the manufacturer's instructions, and subjected to light curing (VALO X LED curing light; Ultradent Products, Inc.).

Fig. 8. Intraoral photographs used for shade matching

A. Photograph taken with a cross-polarization filter and enamel tabs; B. Photograph taken with a cross-polarization filter and dentin tabs; C. Photograph taken with a light source positioned on the palatal side.
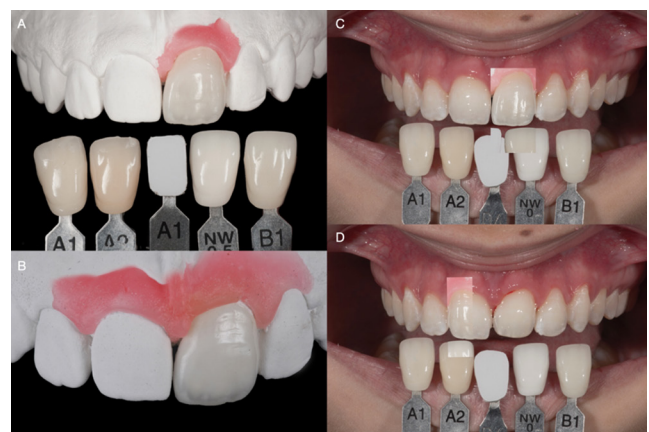

Fig. 9. Restoration and shade matching

A. Feldspathic veneer with shade tabs; B. Tooth restoration; C. Intraoral view for the evaluation of the incisal shade; D. Intraoral view for the evaluation of the interproximal shade.

The restoration was treated with hydrofluoric acid (IPS Ceramic Etching Gel; Ivoclar Vivadent, Schaan, Liechtenstein) for 60 s, followed by rinsing and drying. Then, the restoration was cleaned in an ultrasonic bath (5300 Sweep Ultrasonic Cleaner; Quala Dental Products, Nashville, USA) for 5 min. Silane (Monobond-S; Ivoclar Vivadent) was applied for 60 s, after which the restoration was allowed to air dry. A light-colored resin cement (Variolink Esthetic LC; Ivoclar Vivadent) was applied to the veneer restoration, and the excess was removed with a microbrush and floss (Fig. 10). Light curing was performed for 20 s on the facial, mesial, distal, and incisal surfaces. Subsequently, glycerine gel was applied to prevent the formation of an oxygen inhibition layer, and the surface was light-cured for 20 s. The rubber dam was then removed, and any excess cement at the cervical margin was removed with a surgical scalpel blade (#12 Sterile; Salvin® Dental Specialties, Charlotte, USA).

Fig. 10. Bonding of the final restoration

A. Teflon tape packing; B. Application of the rubber dam; C. Application of the abutment clamp; D. Tooth etching; E. Dental bonding application; F. Veneer placement; G. Cemented restoration.
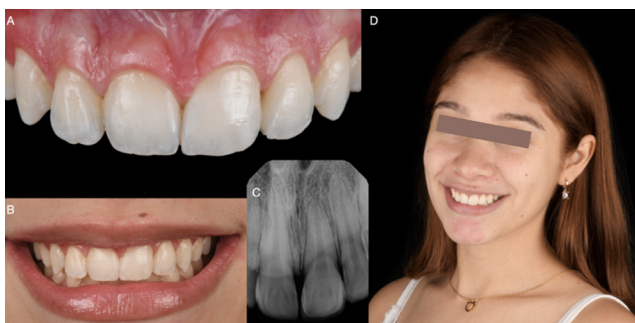

Fig. 11. Final restoration

A. Frontal intraoral view; B. Extraoral close-up; C. Radiographic image; D. Extraoral frontal view.

Static and dynamic occlusion (e.g., incisal guidance and lateral movements) were evaluated. An occlusal night guard was provided to prevent the teeth from wearing down and to protect the new restoration. The patient was satisfied with the results (Fig. 11). At the five-year follow-up, the patient reported continued satisfaction with the clinical outcome (Fig. 12).

Fig. 12. Five-year follow-up

A. Lateral intraoral view; B. Lateral close-up; C. Lateral extraoral view; D. Frontal intraoral view.

Discussion

The patient described in the case report was diagnosed with localized true microdontia, as evidenced by the maxillary left lateral incisor being the only tooth smaller in the arch. Additionally, the radiographs demonstrated smaller roots than those of the adjacent central and lateral incisors, and the patient's facial profile exhibited regular jaw sizes. None of the patient's relatives had any of the most common genetic disorders associated with microdontia. Thus, it can be implied that the development of the dental anomaly may be related to the exposure to environmental factors during the growth and development of the teeth. As previously stated, the etiology of microdontia remains unclear.

The patient described in our report expressed satisfaction with the shade improvements observed after the third whitening appointment and requested to proceed with the restorative stage. The patient evaluated the diagnostic mock-up and approved the proposed restoration of the morphology. The tooth preparation was conducted on the diagnostic mock-up in order to preserve the enamel structure. Following the preparation stage, the remaining mock-up material was removed, and the preparation was polished. Tooth reduction guides were also fabricated on the diagnostic wax-up so that the prosthetic space for the proposed ceramic veneer could be evaluated intraorally. Clinical studies have shown that veneers bonded to enamel have higher long-term success than veneers bonded to dentin.[36,37] The tooth preparation for our patient was completed using an enamel structure.

The selection of tooth shade for the fabrication of a single ceramic restoration in the aesthetic zone is considered one of the most challenging procedures in dentistry. Clinicians can use visual methods and digital devices to record the desired shade.[38] Visual assessment is a traditional and popular technique in which the clinician places shade tabs next to the teeth, with the intention of color matching. The use of shade tabs is a cost-effective and convenient method that depends on human eye observation. However, the disadvantages of shade matching with shade selection include a lack of shade standardization during the manufacturing process of the tabs,[39] light conditions,[40] tooth background,[41] and the clinician's experience.[42,43]

Digital devices used for shade taking include digital cameras, spectrophotometers and spectroradiometers. Digital photography offers a feasible method for shade matching and comparing results before and after treatment under controlled conditions.[44] Spectrophotometers can estimate the shade of a tooth or ceramic object by emitting a light source that, upon reaching the object, is reflected and measured. This process allows for a rapid, accurate and straightforward shade matching.[45] A recent study compared shade matching by visual assessment, cross-polarized photos and spectrophotometry.[46] Similar results were achieved for the cross-polarized photos and spectrophotometry, but there were large differences with the traditional visual assessment.[46] This clinical report used a digital camera to take photos of the prepared tooth with a dark background and a polarizing filter to avoid undesirable surface reflection. Additionally, images of dentin and enamel shade tabs were taken, and photographs with a light background were taken to evaluate fine details, such as craze or microcrack lines. A single feldspathic porcelain veneer with a thickness of 0.6 mm was found to be an appropriate solution for the described patient.

Limitations

The limitations of this brief narrative review include the use of a single database to apply a concise search strategy, which is contrary to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Conclusions

Dental care for patients with focal microdontia may involve the use of adhesive and minimally invasive dentistry techniques since the affected teeth are smaller than usual, and bonding restorations to the enamel structure provide a successful protocol. A single hand-crafted feldspathic porcelain veneer can effectively mimic adjacent teeth, satisfying patients' aesthetic expectations. The additive diagnostic wax-up and intraoral mock-up enable visualization and testing of the aesthetic and functional outcomes of the proposed restoration. Minimally invasive dentistry is an effective treatment option for non-syndromic focal microdontia in young patients, with the potential for long-term high aesthetic results.

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.

ORCID iDs

Carlos Alberto Jurado https://orcid.org/0000-0001-7437-4855
Jose Villalobos-Tinoco https://orcid.org/0000-0003-2156-8335
Abdulrahman Alshabib https://orcid.org/0000-0001-8890-8707
Kelvin Ian Afrashtehfar https://orcid.org/0000-0002-6053-8967

References

1. Thesleff I. Genetic basis of tooth development of dental defects. *Acta Odontol Scand*. 2000;58(5):191–194. doi:10.1080/000163500750051728
2. Osborn JW, Ten Cate AR. *Advanced Dental Histology: Dental Practitioner Handbook No. 6*. Bristol, UK: John Wright & Sons; 1976:24–51.
3. Alexandersen V, Nielsen OV. Generalized microdontia probably associated with intrauterine growth retardation in a medieval skeleton. *Am J Phys Anthropol*. 1970;33(3):389–401. doi:10.1002/ajpa.1330330313
4. Retrouvey JM, Goldberg M, Schwartz S. Dental development and maturation. In: Glorieux FH, Pettifor JM, Jüppner H, eds. *Pediatric Bone*. 2nd ed. Cambridge, MA: Academic Press; 2011:83–108.
5. Chen Y, Zhou F, Peng Y, Chen L, Wang Y. Non-syndromic occurrence of true generalized microdontia with hypodontia: A case report. *Medicine (Baltimore)*. 2019;98(26):e16283. doi:10.1097/MD.0000000000016283
6. Shafer WG, Hine MK, Levy BM. *A Textbook of Oral Pathology*. Philadelphia, PA: W. B. Saunders Company; 1983:26.
7. Villa A, Albonico A, Villa F. Hypodontia and microdontia: Clinical features of a rare syndrome. *J Can Dent Assoc*. 2011;77:b115. PMID:21846459.
8. Küchler EC, Risso PA, de Castro Costa M, Modesto A, Vieira AR. Studies of dental anomalies in a large group of school children. *Arch Oral Biol*. 2008;53(10):941–946. doi:10.1016/j.archoralbio.2008.04.003
9. Saharan K, Shivaprasad S, Ashok L, Shubha C. Microdontia involving mandibular lateral incisor: A rare case report. *Glob J Med Res*. 2018. https://medicalresearchjournal.org/index.php/GJMR/article/view/1609/2-Microdontia-Involving-Mandibular_JATS_NLM_xml#info. Accessed November 20, 2022.
10. Afrashtehfar KI, Assery MKA, Bryant SR. Patient satisfaction in medicine and dentistry. *Int J Dent*. 2020;2020:6621848. doi:10.1155/2020/6621848
11. Jurado C, Watanabe H, Tinoco JV, Valenzuela HU, Guzman Perez G, Tsujimoto A. A conservative approach to ceramic veneers: A case report. *Oper Dent*. 2020;45(3):229–234. doi:10.2341/19-051-T
12. Alikhasi M, Yousefi P, Afrashtehfar KI. Smile design: Mechanical considerations. *Dent Clin North Am*. 2022;66(3):477–487. doi:10.1016/j.cden.2022.02.008
13. Magne P, Magne M. Use of additive waxup and direct intraoral mock-up for enamel preservation with porcelain laminate veneers. *Eur J Esthet Dent*. 2006;1(1):10–19. PMID:19655472.

464

C.A. Jurado et al. Management of focal microdontia

14. Jurado CA, Villalobos-Tinoco J, Tsujimoto A, Castro P, Torrealba Y. The art of minimal tooth reduction for veneer restorations. *Eur J Gen Dent*. 2020;9(1):45–52. doi:10.4103/ejgd.ejgd_173_19

15. Afrashtehfar KI, Assery MK. Five considerations in cosmetic and esthetic dentistry. *J N J Dent Assoc*. 2014;85(4):14–15. PMID:25739136.

16. Yu H, Zhao Y, Li J, et al. Minimal invasive microscopic tooth preparation in esthetic restoration: A specialist consensus. *Int J Oral Sci*. 2019;11(3):31. doi:10.1038/s41368-019-0057-y

17. Horn HR. Porcelain laminate veneers bonded to etched enamel. *Dent Clin North Am*. 1983;27(4):671–684. PMID:6360727.

18. Del Monte S, Afrashtehfar KI, Emami E, Abi Nader S, Tamimi F. Lay preferences for dentogingival esthetic parameters: A systematic review. *J Prosthet Dent*. 2017;118(6):717–724. doi:10.1016/j.prosdent.2017.04.032

19. Öztürk E, Bolay Ş, Hickel R, Ilie N. Shear bond strength of porcelain laminate veneers to enamel, dentine and enamel–dentine complex bonded with different adhesive luting systems. *J Dent*. 2013;41(2):97–105. doi:10.1016/j.jdent.2012.04.005

20. Gurel G, Sesma N, Calamita MA, Coachman C, Morimoto S. Influence of enamel preservation on failure rates of porcelain laminate veneers. *Int J Periodontics Restorative Dent*. 2013;33(1):31–39. doi:10.11607/prd.1488

21. Tsujimoto A, Jurado CA, Villalobos-Tinoco J, et al. Minimally invasive multidisciplinary restorative approach to the esthetic zone including a single discolored tooth. *Oper Dent*. 2021;46(5):477–483. doi:10.2341/20-211-S

22. Jurado CA, Tsujimoto A, Villalobos-Tinoco J, Watanabe H, Takamizawa T, Miyazaki M. Minimally invasive technique for non-vital tooth bleaching using traditional Japanese paper. *J Oral Sci*. 2020;62(4):458–460. doi:10.2334/josnusd.19-0416

23. Tsujimoto A, Jurado CA, Sayed ME, et al. Influence of light irradiation for in-office tooth whitening: A randomized clinical study. *Am J Dent*. 2021;34(4):201–204. PMID:34370912.

24. Watts A, Addy M. Tooth discolouration and staining: A review of the literature. *Br Dent J*. 2001;190(6):309–316. doi:10.1038/sj.bdj.4800959

25. Sulieman M. An overview of tooth discoloration: Extrinsic, intrinsic and internalized stains. *Dent Update*. 2005;32(8):463–464,466–468,471. doi:10.12968/denu.2005.32.8.463

26. Eimar H, Siciliano R, Abdallah MN, et al. Hydrogen peroxide whitens teeth by oxidizing the organic structure. *J Dent*. 2012;40 Suppl 2:e25–e33. doi:10.1016/j.jdent.2012.08.008

27. Pinto MM, Goncalves MLL, Costa da Mota AC, et al. Controlled clinical trial addressing teeth whitening with hydrogen peroxide in adolescents: A 12-month follow-up. *Clinics (Sao Paulo)*. 2017;72(3):161–170. doi:10.6061/clinics/2017(03)06

28. Grahen H. Hypodontia in the permanent dentition: A clinical and genetical investigation. *Odont Rev*. 1956;7:1–100.

29. Thongudomporn U, Freer TJ. Prevalence of dental anomalies in orthodontic patients. *Aust Dent J*. 1998;43(6):395–398. PMID:9973708.

30. Jurado CA, Villalobos-Tinoco J, Mekled S, Sanchez R, Afrashtehfar KI. Printed digital wax-up model as a blueprint for layered pressed-ceramic laminate veneers: Technique description and case report. *Oper Dent*. 2023;48(6):618–626. doi:10.2341/22-144-S

31. Afrashtehfar KI, Pecho OE, El-Mowafy O. How do I manage a patient with a fractured porcelain veneer? *J Can Dent Assoc*. 2015;81:f25. PMID:26679338.

32. Jurado CA, AlResayes S, Sayed ME, Villalobos-Tinoco J, Llanes-Urias N, Tsujimoto A. A customized metal guide for controllable modification of anterior teeth contour prior to minimally invasive preparation. *Saudi Dent J*. 2021;33(7):518–523. doi:10.1016/j.sdentj.2020.09.004

33. McLaren EA, LeSage B. Feldspathic veneers: What are their indications? *Compend Contin Educ Dent*. 2011;32(3):44–49. PMID:21560742.

34. Afrashtehfar KI, Bryant SR. Understanding the lived experience of North American dental patients with a single-tooth implant in the upper front region of the mouth: Protocol for a qualitative study. *JMIR Res Protoc*. 2021;10(6):e25767. doi:10.2196/25767

35. Rabel K, Spies BC, Pieralli S, Vach K, Kohal RJ. The clinical performance of all-ceramic implant-supported single crowns: A systematic review and meta-analysis. *Clin Oral Implants Res*. 2018;29 Suppl 18:196–223. doi:10.1111/clr.13337

36. Oztürk E, Bolay S. Survival of porcelain laminate veneers with different degrees of dentin exposure: 2-year clinical results. *J Adhes Dent*. 2014;16(5):481–489. doi:10.3290/j.jad.a32828

37. Villalobos-Tinoco J, Jurado CA, Afrashtehfar KI, Fischer N. Combination of minimal- and non-preparation techniques with ceramic veneers for managing esthetic deficiencies. *Int J Esthet Dent*. 2023;18(3):232–243. PMID:37462377.

38. Tabatabaian F, Beyabanaki E, Alirezaei P, Epakchi S. Visual and digital tooth shade selection methods, related effective factors and conditions, and their accuracy and precision: A literature review. *J Esthet Restor Dent*. 2021;33(8):1084–1104. doi:10.1111/jerd.12816

39. Cal E, Sonugelen M, Guneri P, Kesercioglu A, Kose T. Application of a digital technique in evaluating the reliability of shade guides. *J Oral Rehabil*. 2004;31(5):483–491. doi:10.1111/j.1365-2842.2004.01197.x

40. Curd FM, Jasinevicius TR, Graves A, Cox V, Sadan A. Comparison of the shade matching ability of dental students using two light sources. *J Prosthet Dent*. 2006;96(6):391–396. doi:10.1016/j.prosdent.2006.10.001

41. Najafi-Abrandabadi S, Vahidi F, Janal MN. Effects of a shade-matching light and background color on reliability in tooth shade selection. *Int J Esthet Dent*. 2018;13(2):198–206. PMID:29687098.

42. Della Bona A, Barrett AA, Rosa V, Pinzetta C. Visual and instrumental agreement in dental shade selection: Three distinct observer populations and shade matching protocols. *Dent Mater*. 2009;25(2):276–281. doi:10.1016/j.dental.2008.09.006

43. Corcodel N, Rammelsberg P, Jakstat H, Moldovan O, Schwarz S, Hassel AJ. The linear shade guide design of Vita 3D-master performs as well as the original design of the Vita 3D-master. *J Oral Rehabil*. 2010;37(11):860–865. doi:10.1111/j.1365-2842.2010.02120.x

44. Hein S, Tapia J, Bazos P. eLABor_aid: A new approach to digital shade management. *Int J Esthet Dent*. 2017;12(2):186–202. PMID:28653050.

45. Posavec I, Prpić V, Zlatarić DK. Influence of light conditions and light sources on clinical measurement of natural teeth color using VITA Easyshade Advance 4,0® spectrophotometer. Pilot study. *Acta Stomatol Croat*. 2016;50(4):337–347. doi:10.15644/asc50/4/7

46. Mahn E, Tortora SC, Olate B, Cacciuttolo F, Kernitsky J, Jorquera G. Comparison of visual analog shade matching, a digital visual method with a cross-polarized light filter, and a spectrophotometer for dental color matching. *J Prosthet Dent*. 2021;125(3):511–516. doi:10.1016/j.prosdent.2020.02.002

Association between glucose metabolism, the circadian cycle and hypoxia: Evaluation of the NPAS2 and Rev-Erb-α protein serum levels in obstructive sleep apnea patients – a pilot study

Filip Franciszek Karuga[1,2,A–F], Julia Jaromirska[1,D–F], Marcin Sochal[1,B,E,F], Piotr Białasiewicz[1,E,F], Agata Gabryelska[1,A–F]

[1] Department of Sleep Medicine and Metabolic Disorders, Medical University of Lodz, Poland

[2] Department of Gynecology, Oncological Gynecology and Treatment of Endometriosis, Polish Mother's Memorial Hospital – Research Institute, Lodz, 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

Address for correspondence
Filip Franciszek Karuga
E-mail: filip.karuga@umed.lodz.pl

Abstract

Background. Obstructive sleep apnea (OSA) is one of the risk factors for diabetes mellitus type 2 (DM2). As OSA is associated with the disruption of the circadian rhythm, it affects circadian clock proteins, including neuronal PAS domain protein 2 (NPAS2) and nuclear receptor subfamily 1 group D member 1 (Rev-Erb-α). These proteins have been shown to be related to metabolic abnormalities, i.a., insulin resistance.

Objectives. The present pilot study aimed to investigate the NPAS2 and Rev-Erb-α protein serum levels in the groups of patients with severe OSA and severe OSA+DM2 in comparison with healthy controls, taking into account correlations with polysomnography (PSG) parameters (e.g., oxygen saturation (SpO$_2$) variables).

Material and methods. A total of 40 participants were included in the study. They were split into 3 groups as follows: the OSA group ($n = 17$; apnea–hypopnea index (AHI) >30, no DM2); the OSA+DM2 group ($n = 7$; AHI > 30 and DM2); and the control group ($n = 16$; AHI < 5, no DM2). All participants underwent a nocturnal PSG examination and had their blood collected the following morning. The serum levels of NPAS2 and Rev-Erb-α proteins were assessed using the enzyme-linked immunosorbent assay (ELISA).

Results. The mean NPAS2 protein level was significantly lower in the OSA group as compared to healthy individuals ($p = 0.017$). Additionally, the OSA group presented with lower NPAS2 protein levels as compared to the OSA+DM2 group, but only a tendency was observed ($p = 0.094$). No differences in the Rev-Erb-α protein concentration were noticed. Furthermore, a negative correlation between AHI during rapid eye movement (REM) sleep and the NPAS2 protein serum level was observed ($r = -0.478$; $p = 0.002$).

Conclusions. Serum NPAS2 protein might be involved in metabolic dysregulation present among OSA patients, while the mechanism itself may be associated with REM sleep.

Keywords: hypoxia, circadian cycle, glucose metabolism, NPAS2, Rev-Erb-α

466

F.F. Karuga et al. NPAS2 and Rev-Erb-α in OSA

Introduction

Obstructive sleep apnea (OSA) is characterized by recurrent pauses in breathing during sleep, leading to intermittent hypoxia, arousal and sleep fragmentation.[1] The gold standard in the OSA diagnosis is a polysomnography (PSG) examination, which enables the assessment of OSA severity through the apnea–hypopnea index (AHI).[2] The available literature recognizes OSA as an independent risk factor for various comorbidities, including glucose metabolism impairment, insulin resistance and diabetes mellitus type 2 (DM2).[3,4] These metabolic complications can be related to electrolyte and vitamin dysregulation,[5] serotoninergic system dysregulation[6–8] or the alteration of circulating adipokines.[9]

Circadian clocks are endogenous coordinators of the 24-hour rhythm of behavioral and molecular processes.[10] Each circadian clock comprises a set of genes that function as activators, namely circadian locomotor output cycles kaput (*CLOCK*) and basic helix–loop–helix ARNT-like (*BMAL1*).[11] Through binding to regulatory elements containing E-boxes, they activate the transcription of repressor proteins period (encoded by *Per*) and cryptochrome (encoded by *Cry*).[12] Neuronal PAS domain protein 2 (NPAS2), similar to circadian clock activators and hypoxia-inducible factors, belongs to the helix–loop–helix Per/Armt/Sim (PAS) transcription factor family. Due to this structural similarity, it can substitute the function of CLOCK.[13] In mice, which serve as a mammalian model of the circadian clock,[14] the activation of CLOCK–BMAL1 occurs in the daytime, which subsequently causes the transcription of both *Per* and *Cry* in the afternoon, and the accumulation of PER and CRY proteins in the evening. The proteins translocate to the nucleus at nighttime and interact with the CLOCK–BMAL1 complex, which results in the repression of their transcription. Following a decrease in the protein concentration at night, the CLOCK–BMAL1 complex starts a new cycle in the morning.[15] The regulatory complex of the circadian clock also includes a supporting loop that involves nuclear receptor subfamily 1 group D member 1 (Rev-Erb-α), which interacts with the CLOCK–BMAL1 complex, taking part in the interlocked feedback loops of activators and repressors, thereby creating a canonical positive- and negative-feedback gene network.[16] Studies have shown that the dysregulation of the circadian clock can result in metabolic complications, including glucose metabolism impairment.[17]

Therefore, this pilot study aimed to investigate the NPAS2 and Rev-Erb-α protein serum levels in patients with severe OSA, severe OSA and DM2, and in healthy individuals, and to assess their relationship with PSG parameters.

Material and methods

This cross-sectional study was conducted at the Department of Sleep Medicine and Metabolic Disorders of the Medical University of Lodz, Poland, between January and June 2021 – recruitment and data collection. Patients were recruited according to the inclusion criteria (age above 18 and below 70 years, body mass index (BMI) of 20–40 kg/m^2, and providing informed written consent to participate in the study). The exclusion criteria comprised withdrawing of the consent, chronic respiratory diseases, chronic inflammatory diseases, infection within 1 month of blood collection, active cancer or a history of cancer, a lifetime history of diagnosed sleep disorders other than OSA, employment in the changing shift system, caffeine intake >900 mg per day, and the use of hypnotic medications or any medications known to affect sleep during 2 weeks before the sleep laboratory examination. Patients referred with suspected OSA syndrome underwent a standard medical examination and a nocturnal PSG examination. The DM2 diagnosis was based on the patient's medical history. After the examination, venous blood samples were collected into serum separator blood collection tubes. Forty participants were qualified for the study. Based on the PSG and clinical data, the participants were assigned to one of 3 study groups: OSA (severe OSA – AHI > 30, no DM2); OSA+DM2 (severe OSA – AHI > 30 and DM2); and control (no OSA – AHI < 5, no DM2). The study was approved by the Bioethical Committee of the Medical University of Lodz (RNN/432/18/KE).

Polysomnography

The patients were admitted to the sleep laboratory at about 9 p.m. (±0.5 h), where standard physical and subjective examinations were conducted, and body mass, height, blood pressure, and pulse were measured. Standard nocturnal PSG was performed by recording the following channels: electroencephalography (EEG) (C4\A1, C3\A2); electromyography (EMG) of the chin muscles and tibialis anterior; electrooculography (EOG); measurements of the oronasal airflow (a thermistor gauge); snoring; body position; respiratory movements of the chest and the abdomen (piezoelectric gauges); unipolar electrocardiogram (ECG); and hemoglobin oxygen saturation (SpO$_2$) (Alice 6; Phillips Respironics, Murrysville, USA). Polysomnographic events were scored by the same experienced physician. Sleep stages were scored according to the criteria based on the 30-second epoch standard.[2]

Biochemical analysis

Peripheral blood samples were collected in the morning following PSG (between 6 a.m. and 7 a.m., within 10 min of awakening) into blood collection tubes with a clot activator and ethylenediaminetetraacetic acid (EDTA).

Blood samples with a clot activator were centrifuged immediately after blood draws at 4°C. Serum was collected and stored at –80°C. The serum protein concentration was assessed using an enzyme-linked immunosorbent assay (ELISA) kit (EIAaB, Wuhan, China) and the absorbance was measured at wavelength λ = 450 nm, using an absorbance reader (BioTek 800 TS; Agilent Technologies Inc., Santa Clara, USA).

Statistical analysis

Statistical analysis was conducted with the use of Statistica 13 PL (StatSoft Polska, Krakow, Poland). The assessment of distribution (normal/non-normal) for continuous variables was performed with the Shapiro–Wilk test. Parametric independent variables were assessed with Student's t test, and the Mann–Whitney U test was used for nonparametric independent variables. Spearman's correlation test was applied for correlations. A p-value <0.05 was considered statistically significant.

Results

The baseline characteristics of the pilot study participants are shown in Table 1, and the results, including the serum levels of NPAS2 and Rev-Erb-α proteins, are presented in Table 2. No statistically significant differences were noticed in the Rev-Erb-α protein concentration among all study groups (p = 0.624). The mean NPAS2 protein level was significantly lower in the OSA group as compared to healthy individuals (p = 0.017). Additionally, the OSA group presented with lower NPAS2 protein levels as compared to the OSA+DM2 group, but only a trend was observed, as the difference did not reach statistical significance (p = 0.094). Of the evaluated correlations, only a weak negative correlation between AHI during rapid eye movement (REM) sleep and the NPAS2 protein serum level was observed (r = –0.478; p = 0.002).

Table 2. Concentrations of circadian clock proteins

Parameter	OSA group (n = 17)	OSA+DM2 group (n = 7)	Control group (n = 16)	p-value
NPAS2 level [ng/mL]	117.07 ±55.29	198.28 ±259.83	186.22 ±166.31	0.037* 0.017*[a] 0.446[b] 0.094[c]
Rev-Erb-α level [ng/mL]	240.93 ±73.46	271.31 ±89.66	272.04 ±92.81	0.624 0.368[a] 0.947[b] 0.505[c]

Data presented as mean ± standard deviation ($M \pm SD$).
NPAS2 – neuronal PAS domain protein 2; Rev-Erb-α – nuclear receptor subfamily 1 group D member 1; * statistically significant ([a] control group vs. OSA group, [b] control group vs. OSA+DM2 group, [c] OSA group vs. OSA+DM2 group).

Table 1. Baseline characteristics of the study population

Parameter		OSA group (n = 17)	OSA+DM2 group (n = 7)	Control group (n = 16)	p-value
Age [years] Me (IQR)		53 (44.50–59.50)	64 (56.00–72.00)	46 (33.75–56.50)	0.003*
Sex n (%)	M	14 (82.35)	6 (85.71)	11 (68.75)	0.548
	F	3 (17.65)	1 (14.29)	5 (31.25)	
BMI [kg/m²] Me (IQR)		33.95 (30.99–37.54)	35.89 (32.08–42.67)	27.33 (24.27–28.88)	<0.001*
TST [h] Me (IQR)		6.50 (6.10–7.08)	5.46 (5.20–6.40)	6.20 (5.70–6.47)	0.050
Arousal index [events/h] Me (IQR)		23.70 (19.60–28.75)	28.10 (20.90–38.60)	12.25 (7.07–17.30)	<0.001*
AHI in REM sleep [events/h] Me (IQR)		38.97 (24.44–53.01)	47.51 (29.14–73.88)	1.64 (0.00–7.70)	<0.001*
AHI in NREM sleep [events/h] Me (IQR)		38.58 (32.33–61.15)	45.66 (35.69–62.89)	1.06 (0.35–1.64)	<0.001*
Total AHI [events/h] Me (IQR)		51.40 (35.9–64.15)	51.70 (45.70–63.40)	1.45 (0.52–3.00)	<0.001*
Desaturation index [events/h] Me (IQR)		50.0 (34.8–76.1)	60.0 (51.2–63.0)	2.0 (1.0–3.0)	<0.001*
SpO₂ during desaturation events [%] Me (IQR)		86.9 (80.5–90.1)	87.0 (83.8–88.0)	91.8 (90.5–93.1)	<0.001*

Me – median; IQR – interquartile range; OSA – obstructive sleep apnea; DM2 – diabetes mellitus type 2; M – male; F – female; BMI – body mass index; TST – total sleep time; AHI – apnea–hypopnea index; REM – rapid eye movement; NREM – non-rapid eye movement; SpO_2 – oxygen saturation; * statistically significant.

Discussion

The dysregulation of circadian clock genes has been shown to cause not only sleep disorders, such as sleep phase syndrome,[18] but also metabolic abnormalities, including metabolic syndrome[19] or insulin resistance.[20] Zhang et al. revealed that diabetic rats had significantly higher Rev-Erb-α protein levels in adipose tissue as compared to nondiabetic rats.[21] Their work suggests that Rev-Erb-α may be one of the key molecules potentially causing metabolic syndromes through circadian clock disruption.[21] Additionally, in a study by Kooner et al., which included 5,561 patients with DM2, *NPAS2* genes were also associated with the development of DM2.[22] Literature concerning circadian clock disruption among patients suffering from OSA is limited, both in terms of number of publications and only several circadian clock genes being investigated.[23] Canales et al., in a cross-sectional study on 49 patients, compared groups with OSA or nocturnal hypoxemia (defined as ≥10% of total sleep time (TST) spent at $SpO_2 < 90\%$) to those without such conditions, and found that the mRNA expression of *Rev-Erb-α* and *NPAS2* was reduced in patients with nocturnal hypoxemia.[24] Nevertheless, the study was a sub-analysis of a cohort suffering from severe kidney dysfunction. Since it has been established that kidney function greatly influences the expression of circadian clock genes,[25] it is impossible to state whether OSA severity, hypoxia or kidney dysfunction had a key impact on the obtained results.

Our results from a pilot study show the same tendency as reported in research by Xie et al., where *NPAS2* gene expression was decreased in untreated OSA patients with AHI > 15.[26] However, their results did not present statistical significance, contrary to our study, where the difference in the NPAS2 protein levels between the OSA group and the control group was significant ($p = 0.017$). Extended research that would include both NPAS2 gene expression and protein level, together with CLOCK–BMAL1 investigations, might help define exact relationships between circadian clock activators in response to hypoxia occurring in the REM phase.

Limitations

Our study has its limitations, including a small sample size (only 40 participants). We aimed to conduct a small-scale study to test the research design and feasibility to obtain significant results, which was achieved. Therefore, we stand prepared to expand the investigation. As the research was conducted as a pilot study and the group of participants was small, it was hard to choose subjects that would perfectly match all parameters; thus, the age of the subjects varied. Another limitation that should be mentioned are the conditions in which the study was conducted, namely sleeping in the hospital PSG laboratory;

TST might have been influenced more by the attitude and the psychological aspects of adjusting to a new sleep environment than by typical circadian-related difficulties in falling asleep.

Conclusions

Serum NPAS2 protein might be involved in metabolic dysregulation present among OSA patients, while the mechanism itself may be associated with REM sleep. The findings of our pilot study may help better understand the molecular mechanism responsible for circadian cycles, the reaction to hypoxia, and their influence on disturbances of glucose metabolism, as well as interactions between them. However, to confirm the role of NPAS2 and Rev-Erb-α in the development of DM2, the continuation of the study is necessary.

Ethics approval and consent to participate

The study was approved by the Bioethical Committee of the Medical University of Lodz, Poland (RNN/432/18/K). All subjects provided informed written consent to participate in the study.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Filip Franciszek Karuga ⬤ https://orcid.org/0000-0002-6551-6804
Julia Jaromirska ⬤ https://orcid.org/0009-0004-6474-5922
Marcin Sochal ⬤ https://orcid.org/0000-0003-2122-0304
Piotr Białasiewicz ⬤ https://orcid.org/0000-0002-8338-1747
Agata Gabryelska ⬤ https://orcid.org/0000-0002-4430-6488

References

1. Lévy P, Kohler M, McNicholas WT, et al. Obstructive sleep apnoea syndrome. *Nat Rev Dis Primers*. 2015;1:15015. doi:10.1038/nrdp.2015.15
2. Kapur VK, Auckley DH, Chowdhuri S, et al. Clinical practice guideline for diagnostic testing for adult obstructive sleep apnea: An American Academy of Sleep Medicine clinical practice guideline. *J Clin Sleep Med*. 2017;13(3):479–504. doi:10.5664/jcsm.6506
3. Hamilton GS, Naughton MT. Impact of obstructive sleep apnoea on diabetes and cardiovascular disease. *Med J Aust*. 2013;199(8):S27–S30. doi:10.5694/mja13.10579
4. Kim T, Kang J. Relationship between obstructive sleep apnea, insulin resistance, and metabolic syndrome: A nationwide population-based survey. *Endocr J*. 2023;70(1):107–119. doi:10.1507/endocrj.EJ22-0280
5. Kanclerska J, Wieckiewicz M, Nowacki D, et al. Sleep architecture and vitamin D in hypertensives with obstructive sleep apnea: A polysomnographic study. *Dent Med Probl*. 2024;61(1):43–52. doi:10.17219/dmp/172243

6. Wieckiewicz M, Martynowicz H, Lavigne G, et al. An exploratory study on the association between serotonin and sleep breathing disorders. *Sci Rep.* 2023;13(1):11800. doi:10.1038/s41598-023-38842-y

7. Choi WG, Choi W, Oh TJ, et al. Inhibiting serotonin signaling through HTR2B in visceral adipose tissue improves obesity-related insulin resistance. *J Clin Invest.* 2021;131(23):e145331. doi:10.1172/JCI145331

8. Yabut JM, Desjardins EM, Chan EJ, et al. Genetic deletion of mast cell serotonin synthesis prevents the development of obesity and insulin resistance. *Nat Commun.* 2020;11(1):463. doi:10.1038/s41467-019-14080-7

9. Lu M, Fang F, Wang Z, Wei P, Hu C, Wei Y. Association between serum/plasma levels of adiponectin and obstructive sleep apnea hypopnea syndrome: A meta-analysis. *Lipids Health Dis.* 2019;18(1):30. doi:10.1186/s12944-019-0973-z

10. Cox KH, Takahashi JS. Circadian clock genes and the transcriptional architecture of the clock mechanism. *J Mol Endocrinol.* 2019;63(4):R93–R102. doi:10.1530/JME-19-0153

11. Gekakis N, Staknis D, Nguyen HB, et al. Role of the CLOCK protein in the mammalian circadian mechanism. *Science.* 1998;280(5369):1564–1569. doi:10.1126/science.280.5369.1564

12. Shearman LP, Sriram S, Weaver DR, et al. Interacting molecular loops in the mammalian circadian clock. *Science.* 2000;288(5468):1013–1019. doi:10.1126/science.288.5468.1013

13. Landgraf D, Wang LL, Diemer T, Welsh DK. NPAS2 compensates for loss of CLOCK in peripheral circadian oscillators. *PLoS Genet.* 2016;12(2):e1005882. doi:10.1371/journal.pgen.1005882

14. Cao F, Ralph MR, Stinchcombe AR. A phenomenological mouse circadian pacemaker model. *J Biol Rhythms.* 2022;37(3):329–342. doi:10.1177/07487304221085455

15. Lowrey PL, Takahashi JS. Genetics of circadian rhythms in mammalian model organisms. *Adv Genet.* 2011;74:175–230. doi:10.1016/B978-0-12-387690-4.00006-4

16. Novák B, Tyson JJ. Design principles of biochemical oscillators. *Nat Rev Mol Cell Biol.* 2008;9(12):981–991. doi:10.1038/nrm2530

17. Malicki M, Karuga FF, Szmyd B, Sochal M, Gabryelska A. Obstructive sleep apnea, circadian clock disruption, and metabolic consequences. *Metabolites.* 2022;13(1):60. doi:10.3390/metabo13010060

18. Xu Y, Padiath QS, Shapiro RE, et al. Functional consequences of a *CKIdelta* mutation causing familial advanced sleep phase syndrome. *Nature.* 2005;434(7033):640–644. doi:10.1038/nature03453

19. Pan X, Mota S, Zhang B. Circadian clock regulation on lipid metabolism and metabolic diseases. *Adv Exp Med Biol.* 2020;1276:53–66. doi:10.1007/978-981-15-6082-8_5

20. Bass J, Takahashi JS. Circadian integration of metabolism and energetics. *Science.* 2010;330(6009):1349–1354. doi:10.1126/science.1195027

21. Zhang R, Yan C, Zhou X, et al. Association of Rev-erbα in adipose tissues with type 2 diabetes mellitus amelioration after gastric bypass surgery in Goto–Kakizaki rats. *Am J Physiol Regul Integr Comp Physiol.* 2013;305(2):R134–R146. doi:10.1152/ajpregu.00520.2012

22. Kooner JS, Saleheen D, Sim X, et al. Genome-wide association study in individuals of South Asian ancestry identifies six new type 2 diabetes susceptibility loci. *Nat Genet.* 2011;43(10):984–989. doi:10.1038/ng.921

23. Yang MY, Lin PW, Lin HC, et al. Alternations of circadian clock genes expression and oscillation in obstructive sleep apnea. *J Clin Med.* 2019;8(10):1634. doi:10.3390/jcm8101634

24. Canales MT, Holzworth M, Bozorgmehri S, et al. Clock gene expression is altered in veterans with sleep apnea. *Physiol Genomics.* 2019;51(3):77–82. doi:10.1152/physiolgenomics.00091.2018

25. Firsov D, Bonny O. Circadian rhythms and the kidney. *Nat Rev Nephrol.* 2018;14(10):626–635. doi:10.1038/s41581-018-0048-9

26. Xie T, Guo D, Luo J, et al. The relationship between *HIF1α* and clock gene expression in patients with obstructive sleep apnea. *Nat Sci Sleep.* 2022;14:381–392. doi:10.2147/NSS.S348580

Dental
and Medical Problems