Dental and Medical Problems

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

2023, Vol. 60, No. 2 (April–June)

Impact Factor (IF) – 2.6
Ministry of Science and Higher Education – 70 pts
Index Copernicus (ICV) – 132.50

Wroclaw Medical University

Dental and Medical Problems

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

QUARTERLY
2023, Vol. 60, No. 2
(April–June)

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

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

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

Online edition is the original version of the journal

Manuscript editing
Joanna Gudarowska
Jolanta Prazeres

Editorial Policy

During the review process, the Editorial Board conforms to the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication" approved by the International Committee of Medical Journal Editors (http://www.icmje.org/). Journal accepts only original papers and all kinds of reviews (narrative review, systematic review, systematic review and meta-analysis, etc.). Experimental studies must include a statement that the experimental protocol and informed consent procedure were in compliance with the Helsinki Convention and were approved by an ethics committee.

Dental and Medical Problems has received financial support from the resources of the Ministry of Science and Higher Education within the "Social Responsibility of Science – Support for Academic Publishing" project based on agreement No. RCN/SP/0493/2021.

Ministerstwo
Edukacji i Nauki

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

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

Dental
and Medical Problems

QUARTERLY 2023, Vol. 60, No. 2 (April–June)

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

Contents

Original papers

201 Mina Biria, Mandana Sattari, Negin Eslamiamirabadi, Atieh Ehsani, Parastoo Iranparvar
Relationship between the salivary concentration of matrix metalloproteinases 8 and 20 and severe early childhood caries

207 Neeta Vijay Bhavsar, Sakshee Trivedi, Kirti Suresh Vachhani, Nilam Brahmbhatt, Shraddha Shah, Neesha Patel, Divya Gupta, Ramya Periasamy
Association between preterm birth and low birth weight and maternal chronic periodontitis: A hospital-based case–control study

219 Selver Suna Basak, Eda Dokumacioglu
Evaluation of the effects of different mouthrinses on dental remineralization

227 Adel Tabesh, Mahboobeh Mahmood, Samin Sirous
Oral health-related quality of life and xerostomia in type 2 diabetic patients

233 Magdalena Piskórz, Karolina Futyma-Gąbka, Ingrid Różyło-Kalinowska
Prevalence of two-rooted and one-rooted mandibular canines with two root canals in Poland, assessed using CBCT: A preliminary study

239 Mohey Aldeen Amam, Anas Abdo, Amirah Alnour, Amam Amam, Mohamad Hassan Jaafo
Comparison of calcium sulfate and tricalcium phosphate in bone grafting after sinus lifting for dental implantation: A randomized controlled trial

247 Soodeh Tahmasbi, Massoud Seifi, Ali Asghar Soleymani, Fatemeh Mohamadian, Mostafa Alam
Comparative study of changes in the airway dimensions following the treatment of Class II malocclusion patients with the twin-block and Seifi appliances

255 Joanna Toczewska, Dagmara Baczyńska, Anna Zalewska, Mateusz Maciejczyk, Tomasz Konopka
The mRNA expression of genes encoding selected antioxidant enzymes and thioredoxin, and the concentrations of their protein products in gingival crevicular fluid and saliva during periodontitis

267 Swatantrata Dey, Anwesh Reddy Nandigam, Anil Kumar Kancharla, Sheema Tasneem Mohammad, Shiva Shankar Gummaluri, Hemalatha Doppalapudi, Anjaneya Mahapatra, Samuel Padala
Scanning electron microscopy study to evaluate and compare fibrin clot adhesion over the root surface treated with tetracycline, doxycycline and minocycline

273 Magdalena Piskórz, Aleksandra Bukiel, Karolina Kania, Dorota Kałkowska, Ingrid Różyło-Kalinowska
Retromolar canal: Frequency in a Polish population based on CBCT and clinical implications – a preliminary study

279 Hirak Shubhra Bhattacharya, Shiva Shankar Gummaluri, Avantika Rani, Satyaki Verma, Preeti Bhattacharya, Shiva Manjunath Rayashettypura Gurushanth
Additional benefits of titanium platelet-rich fibrin (T-PRF) with a coronally advanced flap (CAF) for recession coverage: A case series

287 Judith Patricia Barrera-Chaparro, Sonia Patricia Plaza-Ruíz, Karen Lorena Parra, Magda Quintero, María Del Pilar Velasco, María Carolina Molinares, Catalina Álvarez
Orthodontic treatment need, the types of brackets and the oral health-related quality of life

295 Shurooq Falih Altaie
Tribological, microhardness and color stability properties of a heat-cured acrylic resin denture base after reinforcement with different types of nanofiller particles

303 Gelareh Tajziehchi, Homeira Ansarilari, Kourosh Afshar
Effect of the CAD-CAM and lost-wax framework fabrication techniques on the fracture strength of porcelain in metal-ceramic restorations

311 Cenk Serhan Ozverel, Sevcan Kurtulmus-Yilmaz
Effect of the application of a hydrogen peroxide home bleaching agent on the cytotoxicity of different CAD-CAM restorative materials

321 Beata Dejak, Elżbieta Bołtacz-Rzepkowska
Mechanism of enamel damage in the grooves of molars during mastication

Reviews

327 Umair Shoukat Ali, Kamil Zafar, Rashna Hoshang Sukhia, Mubassar Fida, Aqeel Ahmed
Effect of bonded and removable retainers on occlusal settling after orthodontic treatment: A systematic review and meta-analysis

335 Lissette Cerón, Mishelle Pacheco, Andrés Delgado Gaete, Wilson Bravo Torres, Daniela Astudillo Rubio
Therapies for sleep bruxism in dentistry: A critical evaluation of systematic reviews

345 Ibrahim F. Alshiddi
Survival rate and clinico-radiographic parameters around narrow-diameter dental implants for fixed dental prostheses in the posterior regions: A systematic review

355 Łukasz Natanek, Marcin Krzysztof Adamiecki, Sebastian Kłosek
Motivational interviewing in promoting oral health: A literature review

Original paper

Relationship between the salivary concentration of matrix metalloproteinases 8 and 20 and severe early childhood caries

Mina Biria[1,A,C,E,F], Mandana Sattari[2,A–C,F], Negin Eslamiamirabadi[3,B–D,F], Atieh Ehsani[1,A–D,F], Parastoo Iranparvar[1,B–F]

[1] Department of Pediatric Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Immunology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3] Department of Dental Sciences, Faculty of Dentistry, McGill University, Montreal, Canada

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):201–206

Address for correspondence
Parastoo Iranparvar
E-mail: dr.p.iranparvar@sbmu.ac.ir
Atieh Ehsani
E-mail: Ehsani.atieh@gmail.com

Funding sources
The Research Center of the School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran (grant No. 310/3925).

Conflict of interest
None declared

Acknowledgements
None declared

Received on July 2, 2021
Reviewed on August 18, 2021
Accepted on September 24, 2021

Published online on June 16, 2023

Abstract

Background. Dental caries is initiated through mineral dissolution by bacterial acids and collagen degradation by endogenous proteolytic enzymes, mainly collagenolytic matrix metalloproteinases (MMPs).

Objectives. The present research aimed to evaluate the relationship between severe early childhood caries (S-ECC) and salivary MMP-8 and MMP-20 concentrations.

Material and methods. Fifty children aged 36–60 months were assigned to either the caries-free (control) group or the S-ECC group. Standard clinical examinations were performed, and approx. 1 mL of expectorated unstimulated whole saliva was collected from all participants. In the S-ECC group, the sampling was repeated 3 months after restorative treatment. All samples were analyzed for the salivary concentrations of MMP-8 and MMP-20, using the enzyme-linked immunosorbent assay (ELISA). Statistical analysis employed the *t* test, the Mann–Whitney *U* test, the χ^2 test, Fisher's exact test, and the paired samples *t* test. The level of significance was set at 0.05.

Results. At baseline, the subjects in the S-ECC group presented with significantly elevated levels of MMP-8 as compared to the control group. However, the salivary concentration of MMP-20 did not exhibit a significant difference between the 2 groups. A significant reduction occurred in the levels of MMP-8 and MMP-20 3 months after restorative treatment in the S-ECC group.

Conclusions. The salivary levels of MMP-8 and MMP-20 were significantly affected by dental restorative treatment in children. Furthermore, MMP-8 was observed to be a better indicator of the dental caries status than MMP-20.

Keywords: dental caries, saliva, biomarker, matrix metalloproteinase 8, matrix metalloproteinase 20

Cite as
Biria M, Sattari M, Eslamiamirabadi N, Ehsani A, Iranparvar P. Relationship between the salivary concentration of matrix metalloproteinases 8 and 20 and severe early childhood caries. *Dent Med Probl.* 2023;60(2):201–206. doi:10.17219/dmp/142564

DOI
10.17219/dmp/142564

Introduction

Early childhood caries (ECC) is defined as the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries) or filled tooth surfaces in any primary tooth of a child aged 72 months or younger. However, any sign of smooth surface caries in children younger than 3 years is considered severe early childhood caries (S-ECC). From ages 3 through 5, one or more decayed, missing (due to caries) or filled smooth surfaces in primary maxillary anterior teeth, or a decayed-missing-filled teeth (dmft) score of ≥4 (age 3), ≥5 (age 4) or ≥6 (age 5) constitute S-ECC.[1] Pain is the most immediate consequence of ECC; it can disrupt the everyday activities of the child. The dental treatment of children with ECC is not always feasible due to their lack of cooperation, and sedation or general anesthesia might be required accordingly.[2] This implies the superiority of caries preventive strategies, which can be improved by determining the various factors responsible for the pathogenesis of dental caries.

The saliva makes a major contribution to the pathogenesis of dental caries, which is partly associated with various components of the immune system in its composition. Saliva sampling is a simple, economical and non-invasive method for oral health examinations. Therefore, in the case of oral diseases, the analysis of salivary biomarkers can be considered an appropriate method for an early diagnosis, as well as for the determination of the prognosis and the treatment success.[3–5]

Dentin is a biological composite of hydroxyapatite and organic compounds, with the organic material constituting 30% of its volume. A total of 90 wt% of dentinal organic material is composed of type I collagen, whereas the remaining 10 wt% consists of non-collagenous proteins. Following the dissolution of dentinal mineral content during the bacterial acidic attack, the extracellular matrix (ECM) is degraded by bacterial enzymes and host enzymes, such as matrix metalloproteinases (MMPs).[6,7]

Matrix metalloproteinases are calcium (Ca)-dependent, zinc (Zn)-containing endopeptidases isolated from dentin, the pulp tissue, odontoblasts, whole saliva, and the bacterial plaque. When the saliva penetrates the cavities created by dentinal caries, salivary MMPs might gain direct access to the demineralized dentin.[5,8–11]

Salivary MMP-8 (collagenase 2) can be derived from the gingival crevicular fluid (GCF) or secreted from the salivary glands.[9,12] This enzyme can convert collagen types I, II and III into one-quarter and three-quarter fragments.[13] Matrix metalloproteinase 20 is produced during primary dentinogenesis, becomes trapped in dentin and might be released during the progression of dental caries. Odontoblasts are the primary cell source responsible for the secretion of MMP-20 into the dentinal tubules.[14,15] Matrix metalloproteinase 20 is also released by ameloblasts in their secretion phase during amelogenesis and plays an essential role in the initial stages of enamel development by decreasing the levels of amelogenin.[12] According to the available literature, mild genetic variations in MMP-20 can alter an individual's susceptibility to caries,[14,16] though laboratory studies have shown that MMP-20 cannot degrade collagen types I and II. Therefore, dentin-bound MMP-20 might be involved in the early alteration of the non-collagenous components of the organic matrix during the progression of caries.[12]

Few studies have shown the relationship between the salivary levels of MMPs and dental caries. However, the confirmation of such a relationship by well-designed clinical studies could make the analysis of these salivary biomarkers a useful tool for determining the severity of dental caries and for evaluating the efficacy of caries preventive measures. Furthermore, if a sufficient number of clinical studies confirm the relationship between high concentrations of MMPs and dental caries, the use of MMP inhibitors might be recommended in children with S-ECC, in addition to emphasis on proper oral hygiene and dietary practices. The present research aimed to investigate the relationship between the salivary concentrations of MMP-8 and MMP-20 and S-ECC.

Material and methods

Participants

A total of 50 children aged 36–60 months were included in the study. They were assigned to either the caries-free (control) group (n = 25) or the S-ECC group (n = 25). The sample size was calculated to be 25 in each group based on previous studies, assuming a type-one error $\alpha = 0.05$ ($Z_{1-\alpha/2} = 1.96$) and a type-two error $\beta = 0.10$ (power of 90%) ($Z_{1-\beta} = 1.28$), and the extraction of the parameters $\mu1 = 55$, $\mu2 = 32$, $\sigma1 = 40$, and $\sigma2 = 20$, using an appropriate statistical formula.[17] The participants were selected from among the children referred to the Department of Pediatric Dentistry at Shahid Beheshti University of Medical Sciences, Tehran, Iran, and from randomly selected kindergartens. The inclusion criteria were as follows: the absence of systemic or gingival diseases; no consumption of medications during the previous 2 months; and the absence of any exfoliating primary teeth or erupting permanent teeth at the time of the study.

Each participant was enrolled in the study after their parent read, understood and completed the informed consent document. Also, a questionnaire was completed to examine the parental level of education, and the type (breast milk or formula milk) and duration of child nighttime feeding. The research was designed and performed in accordance with the Declaration of Helsinki,

and was independently reviewed and approved by the Institutional Committee for Ethics in Research at Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RIDS.REC.1395.216).

Clinical examinations

The presence of dental caries was evaluated based on the World Health Organization (WHO) diagnostic criteria.[1] After being dried with sterile gauze, the teeth were clinically examined using disposable dental mirrors and explorers (Shiang Shin Corporation, Taiwan) under sufficient light. Only children with open tooth contacts were included in the study to ensure the absence of interproximal caries. Children without dental caries were assigned to the caries-free group, and those affected by S-ECC were diagnosed according to the American Academy of Pediatric Dentistry definition of S-ECC. The definition includes the presence of one or more decayed, missing (due to caries) or filled smooth surfaces in primary maxillary anterior teeth, or a dmft score of ≥4 at the age of 3 years, ≥5 at the age of 4 years or ≥6 at the age of 5 years.[1] The dmft index was recorded for each child, as was the plaque index (PI), using the simplified oral hygiene index (OHI-S), which consists of the simplified debris (plaque) and calculus indices (DI-S and CI-S). The present study used the index version modified for primary teeth, as defined by Miglani et al.,[18] for the evaluation of the buccal surfaces of second primary molars and central incisors in the upper right and lower left quadrants.[18,19]

Saliva sampling

The children were asked to refrain from eating, drinking, toothbrushing, and using dental floss for 2 h before sampling, which was carried out between 11:00 a.m. and 12:00 a.m. to minimize the effects of the circadian rhythm on the saliva composition. Afterward, approx. 1 mL of unstimulated whole saliva was collected by passive drooling for 5 min. The samples were collected into sterile, capped, pre-chilled microtubes and coded. The microtubes were immediately transferred to the immunology laboratory in a container with dry ice to preserve salivary proteins and prevent their hydrolysis. The saliva samples were centrifuged (Eppendorf® microcentrifuge 5415; Merck, Darmstadt, Germany) at 3,000 g for 15 min. The supernatant from each sample was carefully transferred to a new microtube with a sampler and stored at –80°C until further use.

After completing the required dental treatment and the provision of oral hygiene and nutrition instructions for the patients in the S-ECC group by an experienced post-graduate student of pediatric dentistry, the participants were recalled for post-treatment saliva sampling 3 months later.[5] The salivary samples were collected and stored under the same conditions as outlined above.

Investigating the salivary concentrations of MMP-8 and MMP-20

On the day of the experiment, the microtubes were retrieved from the freezer and kept at room temperature to be thawed. The enzyme-linked immunosorbent assay (ELISA) was performed using human saliva matrix metalloproteinase 8, 20 ELISA kits (Zellbio, Lonsee, Germany). The plates containing the samples were then transferred to an ELISA microplate reader (Anthos 2020; Anthos Mikrosysteme, Friesoythe, Germany) for spectrophotometric analysis at a wavelength of 450 nm. All analyses were performed by 2 experienced immunologists blinded to the sample groups. Inter-examiner reliability was determined using the kappa agreement coefficient ($\kappa = 0.8$). Subsequently, the final salivary concentrations of MMP-8 and MMP-20 were quantified and recorded in nanograms per milliliter (ng/mL).

Statistical analysis

The data was analyzed using IBM SPSS Statistics for Windows, v. 21.0 (IBM Corp., Armonk, USA). The *t* test compared age, the duration of nighttime feeding, PI, and the initial MMP-8 and MMP-20 concentrations between the 2 groups. The Mann–Whitney *U* test compared the dmft scores, and the χ^2 test and Fisher's exact test compared gender distribution, the parental level of education, and the type of nighttime feeding between the 2 groups. Finally, the paired samples *t* test determined the significance of the treatment effect on the salivary concentrations of MMP-8 and MMP-20 in the S-ECC group. The significance level was set at 0.05.

Results

A total of 50 children aged 36–60 months were included in the study, and assigned to either the caries-free ($n = 25$) or S-ECC ($n = 25$) groups. The mean dmft scores in the control and S-ECC groups were 0 and 15.2 ±7.0, respectively. The Mann–Whitney *U* test showed a significant difference between the 2 groups in this regard ($p < 0.001$). Furthermore, the independent *t* test revealed a significant difference between the groups in terms of PI ($p < 0.001$), with the mean values of 0.91 ±0.28 and 1.56 ±0.32 in the control and S-ECC groups, respectively (Table 1).

Table 1. Mean plaque index (PI) values, decayed-missing-filled teeth (dmft) scores, and initial salivary concentrations of matrix metalloproteinases 8 and 20 (MMP-8 and MMP-20) [ng/mL] in the caries-free and severe early childhood caries (S-ECC) groups

Group	PI	dmft	MMP-8	MMP-20
Caries-free	0.91 ±0.28	0	0.336 ±0.047	4.218 ±1.403
S-ECC	1.56 ±0.32	15.2 ±7.0	0.698 ±0.388	3.801 ±0.692

Data presented as mean ± standard deviation (*M* ±*SD*).

The statistical analysis of the data showed no significant differences in age, gender distribution, the parental level of education, and the type of nighttime feeding (breast milk or formula milk) between the 2 groups. However, there was a significant difference in the maternal education levels ($p = 0.010$). The results of the independent t test regarding the duration of nighttime feeding also showed a significant difference between the 2 groups ($p = 0.003$), with the mean duration in the S-ECC group being approx. 6 months longer as compared to the caries-free group.

As presented in Table 1, the independent t test showed that the initial mean salivary concentration of MMP-8 in the S-ECC group was approx. 2 times higher than that in the caries-free group, which was statistically significant ($p < 0.001$). However, there was no significant difference between the 2 groups in the initial mean salivary concentration of MMP-20 ($p = 0.189$). According to the results of the paired samples t test, the salivary concentrations of MMP-8 ($p < 0.001$) and MMP-20 ($p = 0.024$) in the S-ECC group decreased significantly 3 months after restorative treatment (Table 2).

Table 2. Mean pre-treatment and post-treatment (3 months after restorative treatment) salivary concentrations of MMP-8 and MMP-20 [ng/mL] in the S-ECC group

MMP	Saliva sampling	MMP concentration [ng/mL]	*p*-value
MMP-8	pre-treatment	0.698 ±0.388	<0.001*
	post-treatment	0.331 ±0.080	
MMP-20	pre-treatment	3.801 ±0.692	0.024*
	post-treatment	3.438 ±0.309	

Data presented as *M* ±*SD*. * statistically significant (paired samples *t* test; $p < 0.05$ at 95% confidence interval (*CI*)).

Discussion

Salivary components play an essential role in the incidence and development of dental caries due to their continuous contact with the teeth.[20] The degradation of the dentinal organic matrix during the caries process is initiated by endogenous proteolytic enzymes, mainly MMPs.[6,7] Assessing the presence of various MMPs in the saliva by identifying the association between these enzymes and the development or progression of dental caries in children could provide suitable non-invasive diagnostic and prognostic biomarkers.

The present study investigated the salivary concentrations of MMP-8 and MMP-20 in caries-free and S-ECC-affected children, and revealed significantly higher initial salivary concentrations of MMP-8 in the S-ECC group as compared to the caries-free group. In addition, the salivary concentrations of MMP-8 significantly decreased following the completion of dental treatment in the S-ECC group.

There are few clinical studies on the relationship between MMP-8 and dental caries. According to a study by Hedenbjörk-Lager et al., a salivary MMP-8 concentration in adults significantly correlated with the caries rate.[6] In another study by Yang et al., it was concluded that the salivary levels of MMP-8 in children with dental caries were higher than in caries-free individuals.[21] Rabelo Buzalaf et al. reported that individuals with higher salivary concentrations of MMPs were more likely to have dental caries; the authors also showed an inhibitory effect of salivary MMP-8 on the remineralization of the demineralized dentin.[22] Similarly, in another recent study by Ashwini et al., dentin degradation and caries progression were positively correlated with salivary MMP-8 levels.[23] The results of the abovementioned studies are consistent with those of the present study.

The salivary source of MMP-8 is, at least partly, the demineralized decayed dentin.[24] Some amount of MMP-8 remains in the demineralized dentin and is expected to be involved in its degeneration process.[25] Therefore, MMP-8 can be released during the process of the complete or relatively complete degeneration of the demineralized dentin.[6] The MMPs derived from GCF and/or the salivary glands are also involved in the degeneration of the dentinal matrix undergoing full or relative demineralization.[26] Salivary MMPs can gain direct access to dentin through caries-induced cavities.[12]

While considering the effect of caries treatment on the concentrations of MMPs, Chibinski et al. observed an increased expression of MMPs 60 days after sealing the cavity with a glass-ionomer sealant, which is associated with dentin repair rather than the development of caries.[27] The difference between the results of the above study and the present research can be attributed to differences in the treatment procedure, study participants and the experimental method. However, according to another study by the same research group led by Kuhn, sealing the affected dentin of primary molars with glass-ionomer cement reduced the concentration of MMP-8 in dentin, paving the way for the initiation of the repair process by reducing the degenerative effects of this enzyme on the dental tissue.[28] This is consistent with the results of the present research, suggesting a significant decrease in the concentration of MMP-8 in the post-treatment phase. This concentration change is probably due to the reduction of bacterial load following restorative treatment, which significantly decreased the production of chemotactic products and the number of neutrophils in the oral cavity, subsequently leading to a reduction in the concentrations of neutrophil derivatives, such as MMP-8.

The results of the present study showed no significant difference in the initial salivary concentrations of MMP-20 between the 2 groups ($p = 0.189$). However, the concentrations of MMP-20 were significantly reduced in the S-ECC group after the completion of restorative treatment ($p = 0.024$).

Matrix metalloproteinase 20 plays an essential role in the formation of normal enamel, and mutations in this enzyme are related to amelogenesis imperfecta.[16,29] There are few studies on the effect of MMP-20 on dental caries, though Filho et al. reported that the presence of specific MMP-20 genotypes in children was associated with resistance to dental caries.[30] In a recent study by Okamoto et al., MMP-20 was shown to stimulate tertiary dentin formation, facilitating the wound-healing processes in the dentin–pulp complex.[31] Since MMP-20 is found in enamel, the dentinal matrix and the dentinal tubule fluid, the post-treatment reduction in the salivary concentration of this enzyme in the S-ECC group can be attributed to the removal of these sources.[32]

The role of endogenous enzymes of salivary and dentinal origin in the development of dental caries was shown in previous studies.[8,33,34] The current study findings are of great importance, as they propose new ideas for the prevention and treatment of caries. Reducing or preventing the degradation of the organic matrix enables natural lesion repair through remineralization. Mazzoni et al. reported that changes in collagenous and non-collagenous proteins impaired the physical properties of dentin, as well as its remineralization ability.[7] Considering the role of MMPs in collagen degradation, the secretion of these enzymes from the dentin–pulp complex leads to a vicious circle and causes further degeneration of dentinal collagens. Therefore, using MMP inhibitors can be an effective therapeutic intervention for MMP-dependent oral diseases.[7] Several industrial MMP inhibitors have been manufactured, with their inhibitory activity mostly based on their Ca-Zn chelating groups. Ethylenediaminetetraacetic acid (EDTA) is one of the most effective substances.[8] Chlorhexidine can also inhibit MMPs through the Ca-Zn chelation mechanism.[7,9,14]

Considering the limited number of clinical studies on the relationship between MMPs and dental caries, further research should be conducted with larger sample sizes and a more extended follow-up duration.

Conclusions

In conclusion, the results of the present study revealed that dental restorative treatment in children significantly affected salivary MMP-8 and MMP-20 levels. Furthermore, MMP-8 was shown to be a better indicator of the dental caries status than MMP-20.

Ethics approval and consent to participate

The research was designed and performed in accordance with the Declaration of Helsinki, and was independently reviewed and approved by the institutional committee for ethics in research at Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RIDS.REC.1395.216). The informed written consent was obtained from the participants' parents.

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

Mina Biria https://orcid.org/0000-0002-0126-8020
Mandana Sattari https://orcid.org/0000-0001-9839-8528
Negin Eslamiamirabadi https://orcid.org/0000-0001-9345-7111
Atieh Ehsani https://orcid.org/0000-0002-1959-4422
Parastoo Iranparvar https://orcid.org/0000-0001-7071-2124

References

1. Colak H, Dülgergil CT, Dalli M, Hamidi MM. Early childhood caries update: A review of causes, diagnoses, and treatments. *J Nat Sci Biol Med*. 2013;4(1):29–38. doi:10.4103/0976-9668.107257
2. Ximenes M, Cardoso M, Astorga F, Arnold R, Pimenta LA, de Sousa Viera R. Antimicrobial activity of ozone and NaF-chlorhexidine on early childhood caries. *Braz Oral Res*. 2017;31:e2. doi:10.1590/1807-3107BOR-2017.vol31.0002
3. Dean JA. *McDonald and Avery's Dentistry for the Child and Adolescent*. 10th ed. eBook on VitalSource®. Maryland Heights, MO: Mosby/Elsevier Health Sciences; 2015:120–173.
4. Javaid MA, Ahmed AS, Durand R, Tran SD. Saliva as a diagnostic tool for oral and systemic diseases. *J Oral Biol Craniofac Res*. 2016;6(1):66–75. doi:10.1016/j.jobcr.2015.08.006
5. Rathnayake N, Gieselmann DR, Heikkinen AM, Tervahartiala T, Sorsa T. Salivary diagnostics – point-of-care diagnostics of MMP-8 in dentistry and medicine. *Diagnostics (Basel)*. 2017;7(1):7. doi:10.3390/diagnostics7010007
6. Hedenbjörk-Lager A, Bjørndal L, Gustafsson A, et al. Caries correlates strongly with salivary levels of matrix metalloproteinase-8. *Caries Res*. 2015;49(1):1–8. doi:10.1159/000360625
7. Mazzoni A, Tjäderhane L, Checchi V, et al. Role of dentin MMPs in caries progression and bond stability. *J Dent Res*. 2015;94(2):241–251. doi:10.1177/0022034514562833
8. Chaussain C, Boukpessi T, Khaddam M, Tjaderhane L, George A, Menashi S. Dentin matrix degradation by host matrix metalloproteinases: Inhibition and clinical perspectives toward regeneration. *Front Physiol*. 2013;4:308. doi:10.3389/fphys.2013.00308
9. Femiano F, Femiano R, Femiano L, Jamilian A, Rullo R, Perillo L. Dentin caries progression and the role of metalloproteinases: An update. *Eur J Paediatr Dent*. 2016;17(3):243–247. PMID:27759416.
10. Maciejczyk M, Pietrzykowska A, Zalewska A, Knaś M, Daniszewska I. The significance of matrix metalloproteinases in oral diseases. *Adv Clin Exp Med*. 2016;25(2):383–390. doi:10.17219/acem/30428
11. Jain A, Bahuguna R. Role of matrix metalloproteinases in dental caries, pulp and periapical inflammation: An overview. *J Oral Biol Craniofac Res*. 2015;5(3):212–218. doi:10.1016/j.jobcr.2015.06.015
12. Shimada Y, Ichinose S, Sadr A, Burrow MF, Tagami J. Localization of matrix metalloproteinases (MMPs-2, 8, 9 and 20) in normal and carious dentine. *Aust Dent J*. 2009;54(4):347–354. doi:10.1111/j.1834-7819.2009.01161.x
13. Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res*. 2006;69(3):562–573. doi:10.1016/j.cardiores.2005.12.002
14. Tannure PN, Küchler EC, Lips A, et al. Genetic variation in MMP20 contributes to higher caries experience. *J Dent*. 2012;40(5):381–386. doi:10.1016/j.jdent.2012.01.015
15. Sulkala M, Larmas M, Sorsa T, Salo T, Tjäderhane L. The localization of matrix metalloproteinase-20 (MMP-20, enamelysin) in mature human teeth. *J Dent Res*. 2002;81(9):603–607. doi:10.1177/154405910208100905

16. Kim JW, Simmer JP, Hart PS, et al. MMP-20 mutation in autosomal recessive pigmented hypomaturation amelogenesis imperfecta. *J Med Genet*. 2005;42(3):271–275. doi:10.1136/jmg.2004.024505
17. Biria M, Sattari M, Golpayegani MV, Kooshki F. Association of salivary sCD14 concentration levels with early childhood caries. *Iran J Immunol*. 2010;7(3):193–197. PMID:20876990.
18. Miglani DC, Beal JF, James PM, Behari SA. The assessment of dental cleanliness status of the primary dentition using a modification of the simplified oral hygiene index (OHIS-M). *J Indian Dent Assoc*. 1973;45(12):385–388. PMID:4535076.
19. Greene JC, Vermillion JR. The simplified oral hygiene index. *J Am Dent Assoc*. 1964;68:7–13. doi:10.14219/jada.archive.1964.0034
20. Gao X, Jiang S, Koh D, Hsu CYS. Salivary biomarkers for dental caries. *Periodontol 2000*. 2016;70(1):128–141. doi:10.1111/prd.12100
21. Yang TY, Zhou WJ, Du Y, et al. Role of saliva proteinase 3 in dental caries. *Int J Oral Sci*. 2015;7(3):174–178. doi:10.1038/ijos.2015.8
22. Rabelo Buzalaf MA, Hannas AR, Kato MT. Saliva and dental erosion. *J Appl Oral Sci*. 2012;20(5):493–502. doi:10.1590/s1678-77572012000500001
23. Ashwini A, Dineshkumar T, Rameshkumar A, et al. Dentin degradonomics – the potential role of salivary MMP-8 in dentin caries. *J Clin Exp Dent*. 2020;12(2):e108–e115. doi:10.4317/jced.56144
24. Sulkala M, Tervahartiala T, Sorsa T, Larmas M, Salo T, Tjäderhane L. Matrix metalloproteinase-8 (MMP-8) is the major collagenase in human dentin. *Arch Oral Biol*. 2007;52(2):121–127. doi:10.1016/j.archoralbio.2006.08.009
25. Carrilho MR, Tay FR, Donnelly AM, et al. Host-derived loss of dentin matrix stiffness associated with solubilization of collagen. *J Biomed Mater Res B Appl Biomater*. 2009;90(1):373–380. doi:10.1002/jbm.b.31295
26. Tjäderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, Salo T. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. *J Dent Res*. 1998;77(8):1622–1629. doi:10.1177/00220345980770081001
27. Chibinski AC, Gomes JR, Camargo K, Reis A, Wambier DS. Bone sialoprotein, matrix metalloproteinases and type I collagen expression after sealing infected caries dentin in primary teeth. *Caries Res*. 2014;48(4):312–319. doi:10.1159/000355302
28. Kuhn E, Reis A, Campagnoli EB, Rodrigues Chibinski AC, de Oliveira Carrilho MR, Wambier DS. Effect of sealing infected dentin with glass ionomer cement on the abundance and localization of MMP-2, MMP-8, and MMP-9 in young permanent molars in vivo. *Int J Paediatr Dent*. 2016;26(2):125–133. doi:10.1111/ipd.12167
29. Prajapati S, Tao J, Ruan Q, De Yoreo JJ, Moradian-Oldak J. Matrix metalloproteinase-20 mediates dental enamel biomineralization by preventing protein occlusion inside apatite crystals. *Biomaterials*. 2016;75:260–270. doi:10.1016/j.biomaterials.2015.10.031
30. Filho AV, Calixto MS, Deeley K, Santos N, Rosenblatt A, Vieira AR. MMP20 rs1784418 protects certain populations against caries. *Caries Res*. 2017;51(1):46–51. doi:10.1159/000452345.
31. Okamoto M, Takahashi Y, Komichi S, Cooper PR, Hayashi M. Dentinogenic effects of extracted dentin matrix components digested with matrix metalloproteinases. *Sci Rep*. 2018;8(1):10690. doi:10.1038/s41598-018-29112-3
32. McGuire JD, Mousa AA, Zhang BJ, et al. Extracts of irradiated mature human tooth crowns contain MMP-20 protein and activity. *J Dent*. 2014;42(5):626–635. doi:10.1016/j.jdent.2014.02.013
33. Sulkala M, Wahlgren J, Larmas M, et al. The effects of MMP inhibitors on human salivary MMP activity and caries progression in rats. *J Dent Res*. 2001;80(6):1545–1549. doi:10.1177/00220345010800061301
34. Vidal CM, Tjäderhane L, Scaffa PM, et al. Abundance of MMPs and cysteine cathepsins in caries-affected dentin. *J Dent Res*. 2014;93(3):269–274. doi:10.1177/0022034513516979

Original paper

Association between preterm birth and low birth weight and maternal chronic periodontitis: A hospital-based case–control study

Neeta Vijay Bhavsar[A,F], Sakshee Trivedi[B,D,E], Kirti Suresh Vachhani[B,C], Nilam Brahmbhatt[B,E], Shraddha Shah[B], Neesha Patel[B], Divya Gupta[B], Ramya Periasamy[B]

Department of Periodontology, Government Dental College and Hospital, Ahmedabad, India

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):207–217

Address for correspondence
Sakshee Trivedi
E-mail: dr.saksheetrivedi@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
The authors express their sincere gratitude to Prof. Amiya Mehta, Head of the Department of Obstetrics and Gynecology (ObGy) of the Civil Hospital, Ahmedabad, India, for his constant support. The authors also appreciate the help of Assist. Prof. Sujal Parker from the Department of Public Health Dentistry at Government Dental College and Hospital, Ahmedabad, India, with the statistical analysis and the compilation of the results.

Received on April 16, 2022
Reviewed on June 22, 2022
Accepted on July 20, 2022

Published online on June 19, 2023

Cite as
Bhavsar NV, Trivedi S, Vachhani KS, et al. Association between preterm birth and low birth weight and maternal chronic periodontitis: A hospital-based case–control study. *Dent Med Probl.* 2023;60(2):207–217. doi:10.17219/dmp/152234

DOI
10.17219/dmp/152234

Abstract

Background. Periodontal diseases (PDs) are one of the most common chronic diseases affecting overall oral functions, and their association with adverse pregnancy outcomes (APOs) has been an area of interest since the late 90s.

Objectives. The present hospital-based case–control study aimed to find any association between maternal chronic periodontitis (CP) and preterm birth (PTB) and low birth weight (LBW) by comparing the periodontal parameters in patients with normal birth, PTB and LBW.

Material and methods. The participants of the study were females that had delivered a live baby ($n = 1,200$). They were classified as either cases or controls. The cases were defined as PTB if the delivery was before 37 weeks of gestation, and as LBW if the infant weighed <2,500 g. The others were controls. The intraoral examination, which included recording the periodontal status, was conducted within 3 days of delivery. Detailed medical history and demographic data were recorded for the determination of the confounding factors. The multivariable dependence of PTB and LBW on both the categorical and continuous data was analyzed using a multivariate logistic regression analysis. Adjusted odds ratios (*AOR*s) with a 95% confidence interval (*CI*) for the risk of PTB and LBW were calculated.

Results. A strong association with PTB was found for a high plaque index (PI) score (*AOR* = 1.61; $p < 0.001$; 95% *CI*: 1.26–2.07) and a mean pocket probing depth (PPD) ≥4 mm (*AOR*: 4.32; $p < 0.001$; 95% *CI*: 3.09–6.02). A strong association with LBW was found for a high PI score (*AOR* = 2.02; $p < 0.001$; 95% *CI*: 1.43–2.83) and a mean PPD ≥4 mm (*AOR*: 8.70; $p < 0.001$; 95% *CI*: 6.01–12.59). A high PI score and a mean PPD ≥4 mm were independent risk factors for PTB and LBW.

Conclusions. The presence of deep pockets and inadequate plaque control in pregnant females increased the risk of APOs.

Keywords: adverse pregnancy outcomes, low birth weight, maternal chronic periodontitis, preterm birth

Introduction

Periodontitis is amongst the most prevalent chronic diseases globally, affecting approx. 743 million people, with an 11.2% prevalence of severe periodontitis.[1] The majority of this population come from developing countries. Along with dental caries, periodontal diseases (PDs) are the most common chronic diseases affecting overall oral functions and reducing the quality of life; they have been identified as a global epidemic.[2] A Global Burden of Disease (GBD) study revealed an average increase by 45.6% in the prevalence of chronic oral diseases from 1990 to 2010, which is comparable to other non-communicable diseases (NCDs), and adds to the financial burden of the individual.[2] Periodontitis and the major NCDs, like heart disease, diabetes, cancer, and chronic respiratory disease, have common risk factors and social determinants.[1] The association between periodontitis and the abovementioned diseases has been an area of interest since the early 90s.[3,4] Such an association has also been explored in a range of other diseases and conditions, including adverse pregnancy outcomes (APOs), chronic kidney disease, rheumatoid arthritis, dementia, metabolic syndrome, and certain forms of cancer.[2] Many NCDs affect the body in the 3rd and 4th decades of life, but APOs affect newborns. Adverse pregnancy outcomes are a rising concern due to increasing neonatal and infant mortality rates despite medical care and treatment modality advancements.[5] They include preterm birth (PTB), low birth weight (LBW), miscarriage, and early pregnancy loss. Preeclampsia and PTB are major causes of maternal, perinatal and neonatal morbidity and mortality.[6]

Periodontal infection and subsequent inflammation are not limited to the oral cavity. Bacteria and bacterial antigens are systemically dispersed via the pathway of the ulcerated sulcular epithelium[3] and trigger significant systemic inflammation. Leukocytes, endothelial cells and hepatocytes respond to bacteria and virulence factors by secreting pro-inflammatory immune mediators, such as cytokines, chemokines and C-reactive protein (CRP). Soluble antigens from the periodontal inflammatory lesion may enter the bloodstream and react to form a macromolecular complex with the circulating antibodies. These immune complexes can be deposited at various sites in the body and give rise to acute and chronic inflammatory reactions.[7]

During the normal course of pregnancy, as gestation progresses, the amniotic fluid concentrations of prostaglandin E2 (PGE2) and inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin (IL)-1β, rise steadily until reaching a critical threshold level that induces the rupture of the amniotic sac membranes, uterine contractions, cervical dilation, and delivery. The process can be modified by external stimuli, including infection and inflammatory stressors.[8] It has been postulated that the levels of periodontal inflammatory mediators slowly increase and affect the fetomaternal unit.[9] Also, there is evidence for the presence of periodontal pathogens in the amniotic fluid and the placental tissues.[10,11] It is an established fact that there is an increased tendency toward periodontitis during pregnancy due to the effects of hormones,[12] which consequently affects the quality of life of pregnant females.[13] A hospital-based survey revealed a 43% prevalence of periodontitis among pregnant females aged between 19 and 35 years.[14]

To our knowledge, no study investigating the association between APOs and chronic periodontitis (CP) in pregnant females has been published so far. Based on the prevalence of periodontitis in pregnant females and the evidence reviewed above, the present study was conducted in a single hospital[14] to assess the association between CP in pregnant females and APOs.

Material and methods

The present case–control study was conducted in the Department of Obstetrics and Gynecology (ObGy) of the Civil Hospital, Ahmedabad, India. The study was approved by the Institutional Ethics Committee at the Government Dental College and Hospital, Civil Hospital in Ahmedabad (No. IECGDCH/S.10/2017 dated 18/3/2017), the Head of the ObGy Department and the Medical Superintendent of the Civil Hospital. The data was collected from 3 wards of the ObGy Department. The nature and purpose of the study were explained to all the participating patients, and written consent was obtained from all. The study is reported according to the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement.

Study design

The selected period for data collection was between April 2017 and December 2017. There were a total of 6 examiners divided into 3 groups of two, with 1 group assigned to each ward. One examiner reviewed the medical history of all patients who had delivered a live baby, while the other examiner conducted an intraoral examination after obtaining consent and considering patient comfort. All intraoral examinations were done within 72 h of delivery.[4] The 2 appointed examiners per ward were blinded to the patient's medical and dental records, respectively. A total of 1,500 medical records were examined, with 74 females not consenting to participation or a dental checkup and 226 excluded after reviewing their medical records. The data was collected for the remaining 1,200 patients. The prevalence of PTB and/or LBW cases for this population was unknown (Fig. 1).

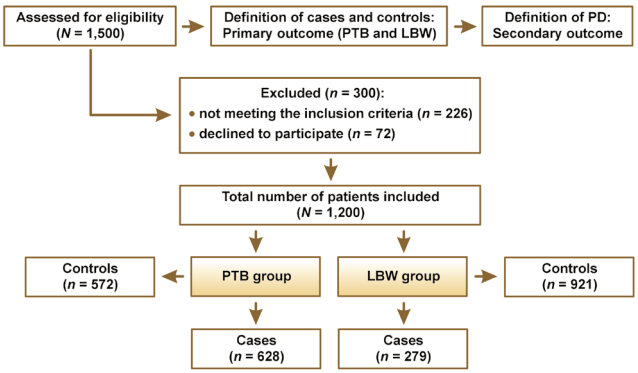

Fig. 1. Patient selection flowchart

PTB – preterm birth; LBW – low birth weight; PD – periodontal disease.

Inclusion criteria

A total of 1,200 females met the inclusion criteria based on medical records and case definition. The patients were aged 18–36 years, had given birth to a live infant and provided consent for an intraoral examination. Three examiners (NP, DG and RP) examined their medical records, and noted the demographic data and medical history, including the patients' conditions during pregnancy and delivery, and the medical records of the infants. An obstetrician reviewed all medical data to determine if the patients met the inclusion or exclusion criteria.

Case definition (primary outcome)

Cases were patients with an infant with (I) PTB or (II) LBW. Preterm birth is defined as childbirth that takes place within 259 days of the mother's last menstrual period (LMP) or 37 completed weeks of gestation, and LBW is an infant birth weight of less than 2,500 g.[15] Controls included patients who had given birth to a live infant after 259 days since the mother's LMP or after 37 completed weeks of gestation, and the infant's birth weight was ≥2,500 g. Gestational age was determined in the 1st trimester through the obstetric criteria based on the date of LMP and/or ultrasound.[16] The information regarding the patient's name, case number, maternal age, socioeconomic status (SES) (modified Kuppuswamy scale),[17] chronic hypertension status, diabetes, urinary tract infection (UTI), parity (number of times a female patient has given birth to a fetus with a gestational age of 24 weeks or more, regardless of whether the child was born alive or stillborn), smoking during pregnancy, drug and/or alcohol use during pregnancy, past gynecological history, as well as the infant's birth weight and gestational age, was collected from the medical records of all the patients who had delivered a live infant. Chronic hypertension was defined as systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg, confirmed by multiple measurements, and detected before conception or 20 weeks of gestation.[18]

Intraoral examination – disease definition (secondary outcome)

The intraoral examination was conducted by 3 qualified examiners, with one appointed to each ward (KSV, NB and SS). Inter and intra-examiner calibration was done before the commencement of the study under the supervision of another researcher (NVB). The examiners were unaware of the patient's medical status, and the infant's birth weight and gestational age. The intraoral examination included recording the plaque index (PI) (Silness and Löe), the gingival index (GI) (Löe and Silness), the pocket probing depth (PPD), and clinical attachment loss (CAL) around all present teeth, excluding third molars. The PPD and CAL were measured at 6 sites of the tooth (midbuccal, buccomesial, buccodistal, midlingual, mesiolingual, and distolingual). The patients were classified as having CP according to the Centers for Disease Control and Prevention and the American Academy of Periodontology (CDC-AAP) definition of periodontitis: no periodontitis – no evidence of mild, moderate or severe periodontitis; mild periodontitis – ≥2 interproximal sites with CAL ≥ 3 mm (not at the same tooth) and ≥2 interproximal sites with PPD ≥ 4 mm (not at the same tooth), or 1 interproximal site with PPD ≥ 5 mm; moderate periodontitis – ≥2 interproximal sites with CAL ≥ 4 mm (not at the same tooth) or ≥2 interproximal sites with PPD ≥ 5 mm (not at the same tooth); and severe periodontitis – ≥2 interproximal sites with CAL ≥ 6 mm (not at the same tooth) and at least 1 interproximal site with PPD ≥ 5 mm.[19,20] On average, 20 patients were examined in each ward, using sterile instruments. The CP15 University of North Carolina probe (UNC-15) (Equinox Instruments Ltd, Lincoln, UK) was used to record GI, PPD and CAL. A data operator simultaneously entered the medical records and the intraoral examination data into a data sheet, matching the case numbers.

Exclusion criteria

Patients were excluded from the study if they had undergone in vitro fertilization, had had multiple gestations, spontaneous abortion in the current gestation, elective and/or induced preterm delivery because of maternal and/or fetal conditions, had any medical condition requiring antibiotic prophylaxis for dental treatment, gestational diabetes, human immunodeficiency virus (HIV) infection, UTI, heart disease, renal disease, hypertensive disorders of pregnancy occurring after 20 weeks of gestation, any dental treatment during the period of gestation, or did not consent to an intraoral examination.

Statistical analysis

After collecting the data, they were compiled, coded and entered into a Microsoft Excel 2019 spreadsheet.

The descriptive analysis was presented as mean (*M*) and standard deviation (*SD*) or as proportions. The proportions between the groups were compared with the χ^2 test. The data distribution was tested using the Kolmogorov–Smirnov test. Based on the normal distribution of the data, Student's *t* test and the Mann–Whitney *U* test were applied to assess differences between the group means. A logistic regression analysis was performed, with PTB and LBW as dependent variables with regard to both the categorical (age, SES, hypertension, diabetes, UTI, previous PTB, PPD < 4 mm or PPD ≥ 4 mm, and CAL: no periodontitis or having periodontitis) and continuous data (PI and GI). A backward stepwise method was used to select independent variables, with an entry at $p = 0.05$ and a removal at $p = 0.10$. Adjusted odds ratios (*AOR*s) with a 95% confidence interval (*CI*) for the risk of PTB and LBW were calculated. All analyses employed IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA). A *p*-value less than 0.05 was considered statistically significant.

Results

There were 628 PTB cases and 279 LBW cases amongst 1,200 live births (Tables 1 and 2, respectively, Fig. 2). The prevalence of PTB was 52.3%, and for LBW it was 23.3%.

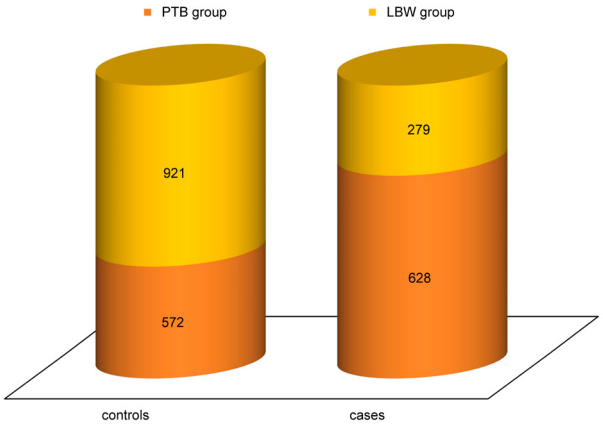

Fig. 2. Distribution of controls and cases in the sample (*N* = 1,200)

There were 258 cases which were both PTB and LBW (Table 1). The mean patient age was 25.09 ±3.19 years. A total of 618 patients had a previous history of PTB – 46.0% in the PTB group and 18.0% in the LBW group (Tables 1 and 2, respectively). The average gestational age was 36.64 ±1.47 weeks (37.75 ±0.54 weeks in the control group and 35.64 ±1.32 weeks in PTB case group) (Table 1). The average infant birth weight was 2,715.07 ±366.06 g, with 2,860.80 ±263.33 g for controls and 2,234.01 ±214.11 g for LBW cases (Table 2).

Table 1. Demographic characteristics of the control and case groups according to gestational age (*N* = 1,200)

Variables		Controls (*n* = 572)	PTB cases (*n* = 628)	Total	*p*-value
Age [years]	18–25	317 (44.4)	397 (55.6)	714	0.020*[a]
	26–30	226 (52.7)	203 (47.3)	429	
	>30	29 (50.9)	28 (49.1)	57	
	M ±*SD*	25.36 ±3.26	24.85 ±3.11	25.09 ±3.19	0.006*[b]
SES	upper (I)	5 (71.4)	2 (28.6)	7	<0.001**[a]
	upper middle (II)	44 (44.0)	56 (56.0)	100	
	middle (III)	298 (54.1)	253 (45.9)	551	
	upper lower (IV)	220 (41.3)	313 (58.7)	533	
	lower (V)	5 (55.6)	4 (44.4)	9	
Hypertension	no	518 (46.6)	594 (53.4)	1,112	0.010*[c]
	yes	54 (61.4)	34 (38.6)	88	
Diabetes	no	549 (47.6)	604 (52.4)	1,153	0.970[c]
	yes	23 (48.9)	24 (51.1)	47	
UTI	no	451 (44.3)	566 (55.7)	1,017	<0.001**[c]
	yes	121 (66.1)	62 (33.9)	183	
LBW	controls	551 (96.33)	370 (58.92)	921(76.75)	<0.001**
	cases	21 (3.67)	258 (41.08)	279 (23.25)	
Previous history of PTB	no	238 (40.9)	344 (59.1)	582	<0.001**[c]
	yes	334 (54.0)	284 (46.0)	618	
Gestational age [weeks] *M* ±*SD*		37.75 ±0.54	35.64 ±1.32	36.64 ±1.47	<0.001**[b]

Data presented as number (percentage) (*n* (%)) or as mean ± standard deviation (*M* ±*SD*). SES – socioeconomic status; UTI – urinary tract infection; * statistically significant ($p < 0.05$); ** highly statistically significant ($p < 0.001$); [a] χ^2 test; [b] Student's *t* test; [c] χ^2 test with the continuity correction.

Table 2. Demographic characteristics of the control and case groups according to the infant birth weight (*N* = 1,200)

Variables		Controls (*n* = 921)	LBW cases (*n* = 279)	Total	*p*-value
Age [years]	18–25	522 (73.1)	192 (26.9)	714	0.001*[a]
	26–30	352 (82.1)	77 (17.9)	429	
	>30	47 (82.5)	10 (17.5)	57	
	M ±*SD*	25.28 ±3.21	24.49 ±3.08	25.09 ±3.19	<0.001**[b]
SES	upper (I)	5 (71.4)	2 (28.6)	7	<0.001**[a]
	upper middle (II)	86 (86.0)	14 (14.0)	100	
	middle (III)	460 (83.5)	91 (16.5)	551	
	upper lower (IV)	363 (68.1)	170 (31.9)	533	
	lower (V)	7 (77.8)	2 (22.2)	9	
Hypertension	no	848 (76.3)	264 (23.7)	1,112	0.190[c]
	yes	73 (83.0)	15 (17.0)	88	
Diabetes	no	883 (76.6)	270 (23.4)	1,153	0.970[c]
	yes	38 (80.9)	9 (19.0)	47	
UTI	no	757 (74.0)	260 (25.0)	1,017	0.620[c]
	yes	164 (89.0)	19 (10.0)	183	
Previous history of PTB	no	414 (71.0)	168 (28.0)	582	<0.001**[c]
	yes	507 (82.0)	111 (18.0)	618	
Infant weight [g] *M* ±*SD*		2,860.80 ±263.33	2,234.01 ±214.11	2,715.07 ±366.06	<0.001**[b]

Data presented as *n* (%) or as *M* ±*SD*. * statistically significant ($p < 0.05$); ** highly statistically significant ($p < 0.001$); [a] χ^2 test; [b] Student's *t* test; [c] χ^2 test with the continuity correction.

Tables 3 and 4 describe the periodontal status of patients in the PTB and LBW groups, respectively. The difference in PI was highly significant, with the score being higher for the PTB ($p < 0.001$) and LBW ($p < 0.001$) groups as compared to controls. The same trend was observed for the GI scores. The mean PPD was 4.79 ±1.28 mm in the PTB group and 4.54 ±1.06 mm in the LBW group, with both values being significantly different when compared with controls ($p < 0.001$). The prevalence of CP was 53.2% in the sample, with 423 cases among the 628 PTB patients and 251 cases among the 279 LBW patients. When compared, the difference between controls and cases was highly significant ($p < 0.001$).The periodontal parameters for the control and case groups according to gestational age and the infant birth weight are graphically presented in Fig. 3,4 and Fig. 5,6, respectively.

Table 3. Periodontal parameters of the control and case groups according to gestational age (*N* = 1,200)

Variables		Controls (*n* = 572)	PTB cases (*n* = 628)	Total	*p*-value
PI		1.47 ±0.57	1.78 ±0.56	1.64 ±0.59	<0.001**[a]
GI		1.77 ±1.85	1.83 ±0.36	1.81 ±1.31	<0.001**[a]
PPD [mm]	<4	499 (59.62)	338 (40.38)	837 (100)	<0.001**[b]
	≥4	73 (20.11)	290 (79.89)	363 (100)	
	M ±*SD*	2.89 ±0.98	4.79 ±1.28	3.23 ±0.71	<0.001**[a]
CP	no periodontitis	357 (63.52)	205 (36.48)	562 (100)	<0.001**[b]
	mild	138 (51.30)	131 (48.70)	269 (100)	
	moderate	37 (26.62)	102 (73.38)	139 (100)	
	severe	40 (17.39)	190 (82.61)	230 (100)	

Data presented as *n* (%) or as *M* ±*SD*. PI – plaque index; GI – gingival index; PPD – pocket probing depth; CP – chronic periodontitis; ** highly statistically significant ($p < 0.001$); [a] Mann–Whitney *U* test; [b] χ^2 test with the continuity correction.

Table 4. Periodontal parameters of the control and case groups according to the infant birth weight (*N* = 1,200)

Variables		Controls (*n* = 921)	LBW cases (*n* = 279)	Total	*p*-value
PI		1.52 ±0.57	1.99 ±0.48	1.64 ±0.59	<0.001**[a]
GI		1.77 ±1.48	1.92 ±0.25	1.81 ±1.31	<0.001**[a]
PPD [mm]	<4	760 (90.80)	77 (9.20)	837 (100)	<0.001**[b]
	≥4	161 (44.35)	202 (55.65)	363 (100)	
	M ±*SD*	3.00 ±1.04	4.54 ±1.06	3.23 ±0.71	<0.001**[a]
CP	no periodontitis	534 (95.02)	28 (4.98)	562 (100)	<0.001**[b]
	mild	221 (82.16)	48 (17.84)	269 (100)	
	moderate	86 (61.87)	53 (38.13)	139 (100)	
	severe	80 (34.78)	150 (65.22)	230 (100)	

Data presented as *n* (%) or as *M* ±*SD*. ** highly statistically significant ($p < 0.001$); [a] Mann–Whitney *U* test; [b] χ^2 test with the continuity correction.

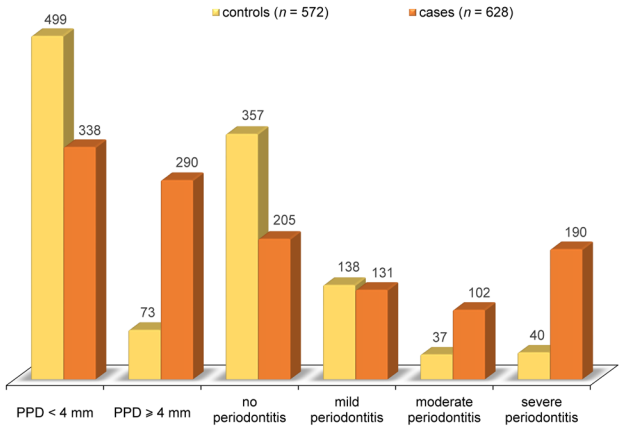

Fig. 3. Distribution of the periodontal parameters in the control and case groups according to gestational age

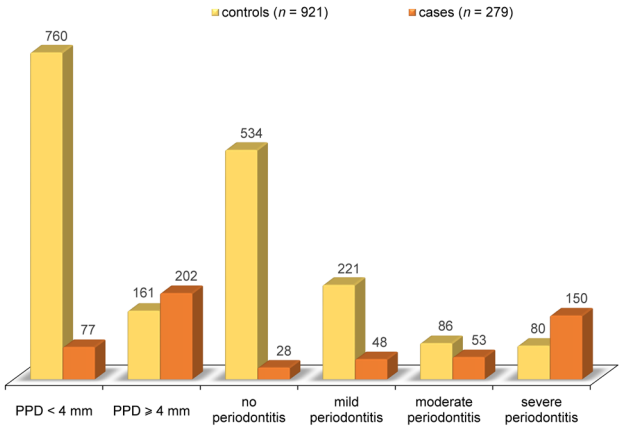

Fig. 5. Distribution of the periodontal parameters in the control and case groups according to the infant birth weight

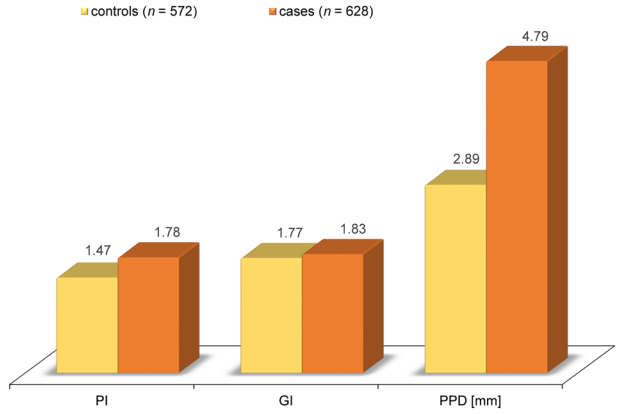

Fig. 4. Periodontal parameters in the control and case groups according to gestational age

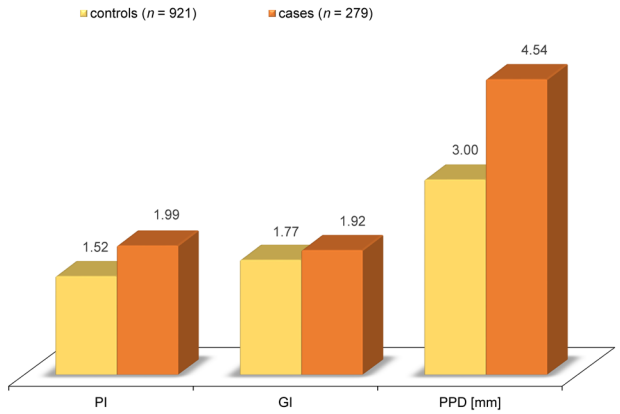

Fig. 6. Periodontal parameters in the control and case groups according to the infant birth weight

Table 5. Logistic regression analysis with preterm birth (PTB) as an outcome variable

Variables		Crude *OR*	*p*-value	95% *CI*	*AOR*	*p*-value	95% *CI*
Age [years]	–	–	0.380	–	–	–	–
	26–30	0.79	0.150	0.59–1.09	–	–	–
	>30	0.89	0.710	0.47–1.67	–	–	–
SES	–	–	0.100	–	–	–	–
	upper middle (II)	6.57	0.050*	0.99–43.49	–	–	–
	middle (III)	4.23	0.130	0.67–26.93	–	–	–
	upper lower (IV)	4.97	0.090	0.78–31.64	–	–	–
	lower (V)	2.24	0.490	0.22–22.86	–	–	–
Hypertension (yes)		0.64	0.070	0.39–1.04	0.63	0.050*	0.39–1.02
Diabetes (yes)		0.92	0.790	0.48–1.74	–	–	–
UTI (yes)		0.45	<0.001**	0.31–0.65	0.46	<0.001**	0.32–0.66
Previous history of PTB (yes)		0.66	0.010*	0.49–0.89	0.59	<0.001**	0.46–0.76
PI		1.76	<0.001**	1.35–2.30	1.61	<0.001**	1.26–2.07
GI		0.92	0.250	0.79–1.06	–	–	–
PPD (≥4 mm)		4.46	0.030*	1.13–17.57	4.32	<0.001**	3.09–6.02
CP (yes)		4.23	<0.001**	2.99–5.98	–	–	–
Constant		0.12	0.030*	–	0.52	0.001*	–

OR – odds ratio; *CI* – confidence interval; *AOR* – adjusted odds ratio; * statistically significant ($p < 0.05$); ** highly statistically significant ($p < 0.001$).

Table 6. Logistic regression analysis with a low birth weight (LBW) as an outcome variable

Variables		Crude *OR*	*p*-value	95% *CI*	*AOR*	*p*-value	95% *CI*
Age [years]	–	–	0.020*	–	–	0.010*	–
	26–30	0.56	0.010*	0.37–0.85	0.55	0.004*	0.36–0.83
	>30	0.53	0.150	0.22–1.27	0.52	0.130	0.22–1.23
SES	–	–	0.430	–	–	–	–
	upper middle (II)	0.66	0.710	0.08–5.79	–	–	–
	middle (III)	0.86	0.880	0.11–6.88	–	–	–
	upper lower (IV)	1.12	0.920	0.14–8.95	–	–	–
	lower (V)	0.61	0.710	0.04–8.69	–	–	–
Hypertension (yes)		0.93	0.830	0.48–1.81	–	–	–
Diabetes (yes)		0.86	0.740	0.36–2.07	–	–	–
UTI (yes)		0.38	0.001*	0.22–0.66	0.37	<0.001**	0.21–0.65
Previous history of PTB (yes)		0.68	0.050*	0.46–0.99	0.68	0.050*	0.47–1.00
PI		2.09	<0.001**	1.47–2.99	2.02	<0.001**	1.43–2.83
GI		0.94	0.540	0.78–1.14	–	–	–
PPD (≥4 mm)		4.16	0.060	0.96–18.09	8.70	<0.001**	6.01–12.59
CP (yes)		7.84	<0.001**	5.33–11.53	–	–	–
Constant		0.06	0.010*	–	0.06	<0.001**	–

* statistically significant ($p < 0.05$); ** highly statistically significant ($p < 0.001$).

Tables 5 and 6 show the results of the logistic regression analysis with regard to risk factors and PTB and LBW, respectively. After the stepwise elimination of insignificant predictors, the factors which had a significant effect on PTB were as follows: a previous history of PTB (*AOR* = 0.59; $p < 0.001$; 95% *CI*: 0.46–0.76); hypertension (*AOR* = 0.63; $p = 0.050$; 95% *CI*: 0.39–1.02); UTI (yes) (*AOR* = 0.46; $p < 0.001$; 95% *CI*: 0.32–0.66); PI (*AOR* = 1.61; $p < 0.001$; 95% *CI*: 1.26–2.07); and PPD (≥4 mm) (*AOR* = 4.32; $p < 0.001$; 95% *CI*: 3.09–6.02) (Table 5). After the stepwise elimination of insignificant predictors, the factors with a significant effect on LBW were as follows: age of 26–30 years (*AOR* = 0.55; $p = 0.004$; 95% *CI*: 0.36–0.83); a previous history of PTB (*AOR* = 0.68; $p = 0.050$; 95% *CI*: 0.47–1.00); UTI (yes) (*AOR* = 0.37; $p < 0.001$; 95% *CI*: 0.21–0.65); PI (*AOR* = 2.02; $p < 0.001$; 95% *CI*: 1.43–2.83); and PPD (≥4 mm) (*AOR* = 8.70; $p < 0.001$; 95% *CI*: 6.01–12.59) (Table 6).

Discussion

The purpose of the present study was to find an association between CP and PTB and LBW in pregnant females admitted to the ObGy Department of the Civil Hospital, Ahmedabad, India. Preterm birth is a major cause of child mortality, and it is associated with emotional and economic burden.[5] According to the national, regional and global estimates of the PTB rates in 2010, with time trends since 1990 for selected countries,[21] India is among the 10 countries of the world with the highest number of the estimated PTB cases, which accounts for 60% of all PTBs globally. There are 3.6 million PTBs in India annually, 303,600 babies do not survive, and most deaths are due to prematurity.[22] According to the results of another hospital-based case–control study conducted in Ahmedabad, India, there was a 30% prevalence of LBW, and 644 out of 1,317 LBW babies were preterm born.[23] In a community-based study designed to identify possible risk factors for PTB in selected districts of Gujarat, India, the proportion of preterm babies out of a total of 2,009 deliveries was 8.9%.[24] The prevalence of PTB (52.3%) and LBW (23.3%) was found to be high in our sample. Despite excluding the confounding factors for PTB and LBW, the high prevalence warrants more in-depth research to find other factors affecting gestation time and the birth weight.

The demographic data of the present study shows that the majority of the included patients belonged to SES III and IV. The modified Kuppuswamy scale is commonly used to measure SES in urban and peri-urban communities, and is based on the educational level, occupation and monthly income of the head of the family.[17] The 2017 update of the scale was used for this sample, as the data collection period was between April 2017 and December 2017. According to this scale, SES III and IV refer to the middle and upper lower classes. Of the 628 PTB patients, 90.13% were from SES III (253; 40.29%) and IV (313; 49.84%), and of the 279 LBW participants, 93.55% were from SES III (91; 32.62%) and IV (170; 60.93%). The Civil Hospital in Ahmedabad is a government multispecialty hospital that provides free treatment to patients below the poverty line and offers affordable treatment charges for patients from all SES classes, which can explain the higher

number of patients from SES III and IV. Adverse socioeconomic factors are associated with an increased risk of PTB[11,25] and LBW.[26] For the present sample, the logistic regression analysis assessing risk factors for PTB showed high *OR*s for SES III (*OR* = 4.23; 95% *CI*: 0.67–26.93) and SES IV (*OR* = 4.97; 95% *CI*: 0.78–31.62), though the result was not significant. Moreover, SES as a risk factor did not affect PTB or LBW in this sample, perhaps due to the public health measures taken by the government to improve female reproductive health. The initiated integrated programs have made medical care accessible to females, especially those with a low SES,[27,28] which has helped considerably to reduce maternal malnutrition and improved public awareness in recent years. When considering the results for SES II, it was found to affect PTB (*OR* = 6.57; p = 0.050; 95% *CI*: 0.99–43.49). Since the sample size in SES II was 100 and included 56 PTB patients, and the difference was significant, it warrants further research with regard to SES II affecting PTB.

Another factor that was found to affect the incidence of PTB and LBW was maternal age. The incidence increases in the extremes of female reproductive life, i.e., less than 18 and more than 36 years of age.[11] In the present sample, more patients were aged less than 30 years, which can be attributed to the social and cultural practices followed by the rural and semi-urban population residing in the areas around Ahmedabad. The patients in the 26–30 age group were less likely to have a baby with LBW (*AOR* = 0.55; p = 0.004; 95% *CI*: 0.36–0.83) as compared to other age groups.

Out of 1,200 patients, 618 had a history of PTB, with 284 in the PTB group and 111 in the LBW group. The patients with a history of PTB had a reduced chance of having PTB (*AOR* = 0.59; $p < 0.001$; 95% *CI*: 0.46–0.76) and LBW (*AOR* = 0.68; $p = 0.050$; 95% *CI*: 0.47–1.00). However, other studies found previous PTB to be a significant risk factor for PTB and LBW,[16,29,30] since they obtained higher *OR*s than the current study. According to Meis et al., previous PTB is a significant predictor of PTB.[31] There were 258 (41.08%) cases of LBW in the PTB group. The composite term PTLBW is no longer used in the obstetrics literature, as it may incorporate diversified underlying etiologies.[32] Therefore, this factor was not included in the regression analysis.

Chronic hypertension significantly affects APOs, as confirmed by a meta-analysis of 55 studies that included 795,221 pregnancies, spanning 4 decades.[33] Urinary tract infection during pregnancy is a frequent finding and is an independent risk factor for APOs.[34] During pregnancy, immunological changes help to promote tolerance to the fetus and protect the mother against infection. However, females are more susceptible to infection and the effects of the toxins produced by microorganisms.[34] According to a study published in 2008, asymptomatic bacteriuria is one of the most common causes of preterm labor.[35] A possible explanation for this finding could be that UTI may increase the release of inflammatory chemokines and cytokines, which can stimulate uterine contractions, cause the premature rupture of membranes and induce PTB.[35] In the present sample, there were 88 patients with hypertension, though the presence of hypertension reduced the risk of PTB (*AOR* = 0.63; p = 0.050; 95% *CI*: 0.39–1.02). Furthermore, UTI reduced the risk of PTB (*AOR* = 0.46; $p < 0.001$; 95% *CI*: 0.32–0.66) and LBW (*AOR* = 0.37; $p < 0.001$; 95% *CI*: 0.21–0.65). These results warrant an in-depth study of the effects of these factors on pregnant females.

The overall prevalence of CP in the sample was 53.17% – 67.36% in the PTB group and 89.96% in the LBW group. The first clinical study on the association between APOs and the periodontal status in humans was a case–control study conducted by Offenbacher et al. in 1996.[4] Since then, numerous studies have evaluated the association between maternal periodontal infection and APOs, and many systematic reviews and meta-analyses have also evaluated and discussed the link in detail.[6,15,36,37] In a study conducted in the same hospital, the prevalence of CP in patients aged between 19 and 35 years was 43%, and 41.1% had poor oral hygiene.[14] These discrepancies can be explained by the definition of CP. The study by Gupta et al.[14] used Russell's periodontal index to categorize periodontitis, whereas the present study classified CP using the criteria described in the 'Material and methods' section, with only CAL used to define and classify CP severity.

The differences in the PI and GI scores between the control and the PTB and LBW groups were highly significant, with higher scores among cases. The mean PPD in the PTB group (4.79 ±1.28 mm) was significantly higher than in controls. Of 363 cases, 290 had a mean PPD ≥4 mm. The mean PPD in the LBW group (4.54 ±1.06 mm) was significantly higher than in controls. Out of 363 cases, 202 had a mean PPD ≥4 mm. When CP severity was compared between controls and PTB and LBW cases, a higher number of PTB cases had moderate and severe CP, and more LBW cases had severe CP, with the differences being statistically significant.

Approximately 30–40% of preterm delivery cases are thought to be due to different infections.[10] The possible mechanisms by which periodontal infection affect APOs include the translocation of periodontal pathogens to the feto-placental unit (metastatic infection),[7] and the effects of inflammatory mediators, such as IL-1, IL-6, IL-8, TNF-α, and PGE2, on the feto-placental unit (metastatic inflammation).[7,38] According to a systematic review, patients with APOs had higher levels of inflammatory mediators in GCF.[39] In addition, a cross-sectional study including 120 females (60 normal births and 60 PTBs) concluded that those with PTB demonstrated worse periodontal parameters and significantly increased GCF levels of IL-6 and PGE2 as compared to normal births.[9] In the present study, inflammatory markers were not evaluated with regard to PTB, LBW and normal births.

The human placenta is known to have its own specific microbiome, yet many studies have also found evidence for the presence of periodontal pathogens, such as *Porphyromonas gingivalis*, in it.[38,40] Furthermore, there is a tendency toward pregnancy gingivitis during pregnancy, as gingival tissues are sensitive to hormonal changes and the slightest increase in plaque accumulation can cause an exaggerated response. However, gingivitis during pregnancy is not clinically or histologically different from gingivitis in non-pregnant persons. González-Jaranay et al. found an increase in the PI (O'Leary) and GI (Ainamo and Bay) scores during pregnancy in females who reached a full term, as well as a slight increase after delivery.[41] Radnai et al. also demonstrated higher mean PI scores in the study group (0.79) than in the control group (0.67), though the difference was not significant ($p = 0.141$).[42]

For the present study, PPD ≥ 4 mm was considered one of the parameters of a gingival response to plaque accumulation. According to Lindhe et al., the optimal response to periodontal surgery was observed in pockets deeper than 4 mm; also during the maintenance phase, high plaque scores were noticed in patients with residual pockets greater than 4 mm.[43] Therefore, 4 mm was considered to be the critical PPD value. Patients with a mean PPD ≥4 mm had at least a few pockets deeper than 4 mm. This may have led to more plaque accumulation, which is a critical factor for eliciting a systemic inflammatory response. A reasonable biological model explaining the relationship between periodontitis and other diseases is that periodontitis causes inflammatory burden through bacteremia, which elicits a systemic inflammatory response or cross-reactivity, resulting in an autoimmune reaction. If the surface area of the pocket is calculated, a minimum area of 50 cm^2 allows the penetration of bacterial products into host tissues.[42] Hence, it can be interpreted as the larger the amount of the inflamed periodontal tissue (i.e., the deeper the periodontal pockets), the greater the chance of periodontitis eliciting a systemic response. As such, the periodontal inflamed surface area (PISA) may be considered the main contributor to any systemic inflammatory burden posed by periodontitis.[44] In the present sample, the high PI scores in the PTB (*AOR* = 1.61; $p < 0.001$; 95% *CI*: 1.26–2.07) and LBW (*AOR* = 2.02; $p < 0.001$; 95% *CI*: 1.43–2.83) groups, as well as PPD ≥ 4 mm in the PTB (*AOR* = 4.32; $p < 0.001$; 95% *CI*: 3.09–6.02) and LBW (*AOR* = 8.70; $p < 0.001$; 95% *CI*: 6.01–12.59) groups, significantly increased the risk of PTB and LBW, and were significant predictors of PTB and LBW after the stepwise elimination of insignificant predictors.

In the current sample, the presence of CP significantly increased the risk of PTB (*OR* = 4.23; $p < 0.001$; 95% *CI*: 2.99–5.98) and LBW (*OR* = 7.84; $p < 0.001$; 95% *CI*: 5.33–11.53), though it was eliminated as an independent risk factor after adjustment. An explanation for this observation is that CAL may not be the most appropriate clinical assessment measure when the goal is to assess periodontitis as a cause of the systemic inflammatory responses associated with systemic diseases or conditions.[45] Indeed, CAL results from the chronic inflammatory infection and host response caused by the plaque microorganisms harbored in deep pockets. Hence, the observations of this sample divert attention toward the systemic effects of a local inflammatory response instead of the disease as a singular entity.

López et al. found a significant association between PD and PTB (*RR* (risk ratio) = 3.5; $p = 0.006$; 95% *CI*: 1.30–9.10), and PD to be the only risk factor for LBW (*RR* = 3.5; $p = 0.028$; 95% *CI*: 1.06–11.4).[46] Radnai et al. studied the association between early periodontitis and PTB, and found that patients with periodontitis had a 5.46 times greater chance of preterm delivery or LBW than periodontally healthy females.[42] Siqueira et al. showed a risk association between CP and PTB, LBW and intrauterine growth restriction (IUGR).[16] Khader et al. assessed the association between PD severity and PTB and LBW among females in Northern Jordan, and found that the *OR*s associated with a 1-millimeter increase in the mean PPD and CAL were 2.04 (95% *CI*: 1.59–2.61) and 2.21 (95% *CI*: 1.66–3.00), respectively.[47] Gesase et al. found that PD was significantly associated with LBW (*AOR* = 2.41; 95% *CI*: 1.34–4.33) and PTB (*OR* = 2.32; 95% *CI*: 1.33–4.27).[48] On the other hand, many studies included in a recent systematic review and meta-analysis reported that periodontitis was not a risk factor for PTB or LBW.[49] However, they concluded that PD significantly increased the risk of APOs.[49] Nonetheless, large prospective, blinded cohort studies with standardized PD diagnostic criteria and the adequate control of the confounding factors are still required to confirm the relationship between PD and AOPs.

A systematic review and meta-analysis by Manrique-Corredor et al. showed that 60% of the assessed studies observed a positive association between maternal periodontitis and PTB.[50] They also found heterogeneity in the definition of CP. Researchers have compared the different definitions used to diagnose CP, slightly underestimating the extent of the disease and obtaining higher *OR*s, though *OR*s decreased when the criteria became stricter. Clinical attachment loss, one of the parameters for classifying periodontitis severity and extent, is the measure of the total disease and not of the current disease activity.[42] Given that the present study found a high PI score and a mean PPD ≥4 mm to be independent risk factors, even a shallow pocket is a source of microorganisms and would account for the total inflammatory burden.[44] However, it can be evaluated further by conducting microbiological investigations and mapping the levels of inflammatory mediators in future studies, this being a limitation of the present study.

Conclusions

The present study found that a high PI score and a mean PPD ≥4 mm significantly increased the risk of PTB and LBW. Even though the prevalence of PTB/LBW is higher in the extremes of reproductive age, the present study found a high prevalence of PTB/LBW in the <36 age group. More efforts can be made to study the association between CP (CAL) severity and PTB and LBW in a larger sample.

Ethics approval and consent to participate

The study was approved by the Institutional Ethics Committee at the Government Dental College and Hospital, Civil Hospital in Ahmedabad (No. IECGDCH/S.10/2017 dated 18/3/2017), the Head of the ObGy Department and the Medical Superintendent of the Civil Hospital. The informed written consent was obtained from all participants.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Neeta Vijay Bhavsar https://orcid.org/0000-0002-4379-3376
Sakshee Trivedi https://orcid.org/0000-0002-0294-2179
Kirti Suresh Vachhani https://orcid.org/0000-0002-0358-0712
Nilam Brahmbhatt https://orcid.org/0000-0002-0772-9027
Shraddha Shah https://orcid.org/0000-0001-5319-5153
Neesha Patel https://orcid.org/0000-0001-8851-2999
Divya Gupta https://orcid.org/0000-0003-0858-7363
Ramya Periasamy https://orcid.org/0000-0002-9095-1795

References

1. Tonetti MS, Jepsen S, Jin L, Otomo-Corgel J. Impact of the global burden of periodontal diseases on health, nutrition and well-being of mankind: A call for global action. *J Clin Periodontol*. 2017;44(5):456–462. doi:10.1111/jcpe.12732
2. Jin LJ, Lamster IB, Greenspan JS, Pitts NB, Scully C, Warnakulasuriya S. Global burden of oral diseases: Emerging concepts, management and interplay with systemic health. *Oral Dis*. 2016;22(7):609–619. doi:10.1111/odi.12428
3. Williams RC, Offenbacher S. Periodontal medicine: The emergence of a new branch of periodontology. *Periodontol 2000*. 2000;23:9–12. doi:10.1034/j.1600-0757.2000.2230101.x
4. Offenbacher S, Katz V, Fertik G, et al. Periodontal infection as a possible risk factor for preterm low birth weight. *J Periodontol*. 1996;67(Suppl 10S):1103–1113. doi:10.1902/jop.1996.67.10s.1103
5. Blencowe H, Cousens S, Chou D, et al. Born too soon: The global epidemiology of 15 million preterm births. *Reprod Health*. 2013;10(Suppl 1):S2. doi:10.1186/1742-4755-10-S1-S2
6. Xiong X, Buekens P, Fraser WD, Beck J, Offenbacher S. Periodontal disease and adverse pregnancy outcomes: A systematic review. *BJOG*. 2006;113(2):135–143. doi:10.1111/j.1471-0528.2005.00827.x
7. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. *Clin Microbiol Rev*. 2000;13(4):547–558. doi:10.1128/CMR.13.4.547
8. Madianos PN, Bobetsis YA, Offenbacher S. Adverse pregnancy outcomes (APOs) and periodontal disease: Pathogenic mechanisms. *J Periodontol*. 2013;84(4 Suppl):S170–S180. doi:10.1902/jop.2013.1340015
9. Perunovic ND, Rakic MM, Nikolic LI, et al. The association between periodontal inflammation and labor triggers (elevated cytokine levels) in preterm birth: A cross-sectional study. *J Periodontol*. 2016;87(3):248–256. doi:10.1902/jop.2015.150364
10. Haram K, Seglem Mortensen JH, Wollen AL. Preterm delivery: An overview. *Acta Obstet Gynecol Scand*. 2003;82(8):687–704. doi:10.1034/j.1600-0412.2003.00218.x
11. Williams CE, Davenport ES, Sterne JA, Sivapathasundaram V, Fearne JM, Curtis MA. Mechanisms of risk in preterm low-birthweight infants. *Periodontol 2000*. 2000;23:142–150. doi:10.1034/j.1600-0757.2000.2230115.x
12. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. *Int J Health Sci (Qassim)*. 2017;11(2):72–80. PMID:28539867. PMCID:PMC5426403.
13. Acharya S, Bhat PV. Oral-health-related quality of life during pregnancy. *J Public Health Dent*. 2009;69(2):74–77. doi:10.1111/j.1752-7325.2008.00104.x
14. Gupta D, Bhavsar NV, Trivedi S. Prevalence of periodontitis in pregnant patients attending gynaecology department of government hospital, Ahmedabad. *Int J Curr Res*. 2018;10(10):74738–74741. doi:10.24941/ijcr.32859.10.2018
15. Chambrone L, Guglielmetti MR, Pannuti CM, Chambrone LA. Evidence grade associating periodontitis to preterm birth and/or low birth weight: I. A systematic review of prospective cohort studies. *J Clin Periodontol*. 2011;38(9):795–808. doi:10.1111/j.1600-051X.2011.01755.x
16. Siqueira FM, Miranda Cota LO, Costa JE, Amaral Haddad JP, Quintão Lana ÂM, Costa FO. Intrauterine growth restriction, low birth weight, and preterm birth: Adverse pregnancy outcomes and their association with maternal periodontitis. *J Periodontol*. 2007;78(12):2266–2276. doi:10.1902/jop.2007.070196
17. Singh T, Sharma S, Nagesh S. Socio-economic status scales updated for 2017. *Int J Res Med Sci*. 2017;5(7):3264-3267. doi:10.18203/2320-6012.ijrms20173029
18. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. *Am J Obstet Gynecol*. 2000;183(1):s1–s22. doi:10.1067/mob.2000.107928
19. Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ. Update of the case definitions for population-based surveillance of periodontitis. *J Periodontol*. 2012;83(12):1449–1454. doi:10.1902/jop.2012.110664
20. Holtfreter B, Albandar JM, Dietrich T, et al.; Joint EU/USA Periodontal Epidemiology Working Group. Standards for reporting chronic periodontitis prevalence and severity in epidemiologic studies: Proposed standards from the Joint EU/USA Periodontal Epidemiology Working Group. *J Clin Periodontol*. 2015;42(5):407–412. doi:10.1111/jcpe.12392
21. Lee AC, Katz J, Blencowe H, et al.; CHERG SGA-Preterm Birth Working Group. National and regional estimates of term and preterm babies born small for gestational age in 138 low-income and middle-income countries in 2010. *Lancet Glob Health*. 2013;1(1):e26–e36. doi:10.1016/S2214-109X(13)70006-8
22. Dhabhai N. Preterm birth and periodontal disease: A medical perspective. *J Int Clin Dent Res Organ*. 2016;8(2):98–101. doi:10.4103/2231-0754.186421
23. Mavalankar DV, Gray RH, Trivedi CR. Risk factors for preterm and term low birthweight in Ahmedabad, India. *Int J Epidemiol*. 1992;21(2):263–272. doi:10.1093/ije/21.2.263
24. Trivedi P, Saxena D, Puwar T, Savaliya S, Ganguly P. A cohort study on risk factors for preterm births in rural Gujarat. *Indian J Public Health*. 2018;62(2):111–116. doi:10.4103/ijph.IJPH_337_16
25. Peacock JL, Bland JM, Anderson HR. Preterm delivery: Effects of socioeconomic factors, psychological stress, smoking, alcohol, and caffeine. *BMJ*. 1995;311(7004):531–535. doi:10.1136/bmj.311.7004.531
26. Valero De Bernabé J, Soriano T, Albaladejo R, et al. Risk factors for low birth weight: A review. *Eur J Obstet Gynecol Reprod Biol*. 2004;116(1):3–15. doi:10.1016/j.ejogrb.2004.03.007
27. Ministry of Health and Family Welfare, Government of India. RMNCH+A. https://nhm.gov.in/index1.php?lang=1&level=1&sublinkid=794&lid=168. Accessed June 29, 2021.

28. Jain N. 7 ways public health in India has changed over the last decade. https://www.path.org/articles/7-ways-public-health-india-has-changed. Accessed June 29, 2021.
29. Bassani DG, Olinto MT, Kreiger N. Periodontal disease and perinatal outcomes: A case–control study. *J Clin Periodontol*. 2007;34(1):31–39. doi:10.1111/j.1600-051X.2006.01012.x
30. Silveira da Mota Krüger M, Casarin RP, Dos Santos Pinto G, et al. Maternal periodontal disease and adverse perinatal outcomes: Is there an association? A hospital-based case–control study. *J Matern Fetal Neonatal Med*. 2019;32(20):3401–3407. doi:10.1080/14767058.2018.1464554
31. Meis PJ, Goldenberg RL, Mercer BM, et al. The preterm prediction study: Risk factors for indicated preterm births. Maternal-Fetal Medicine Units Network of the National Institute of Child Health and Human Development. *Am J Obstet Gynecol*. 1998;178(3):562–567. doi:10.1016/s0002-9378(98)70439-9
32. Ide M, Papapanou PN. Epidemiology of association between maternal periodontal disease and adverse pregnancy outcomes – systematic review. *J Clin Periodontol*. 2013;40(Suppl 14):S181–S194. doi:10.1111/jcpe.12063
33. Bramham K, Parnell B, Nelson-Piercy C, Seed PT, Poston L, Chappell LC. Chronic hypertension and pregnancy outcomes: Systematic review and meta-analysis. *BMJ*. 2014;348:g2301. doi:10.1136/bmj.g2301
34. Mazor-Dray E, Levy A, Schlaeffer F, Sheiner E. Maternal urinary tract infection: Is it independently associated with adverse pregnancy outcome? *J Matern Fetal Neonatal Med*. 2009;22(2):124–128. doi:10.1080/14767050802488246
35. Roozbeh N, Moradi S, Soltani S, Zolfizadeh F, Hasani MT, Yabandeh AP. Factors associated with preterm labor in Hormozgan province in 2013. *Electron Physician*. 2016;8(9):2918–2923. doi:10.19082/2918
36. Agueda A, Echeverría A, Manau C. Association between periodontitis in pregnancy and preterm or low birth weight: Review of the literature. *Med Oral Patol Oral Cir Bucal*. 2008;13(9):E609–E615. PMID:18758408.
37. Daalderop LA, Wieland BV, Tomsin K, et al. Periodontal disease and pregnancy outcomes: Overview of systematic reviews. *JDR Clin Transl Res*. 2018;3(1):10–27. doi:10.1177/2380084417731097
38. Komine-Aizawa S, Aizawa S, Hayakawa S. Periodontal diseases and adverse pregnancy outcomes. *J Obstet Gynaecol Res*. 2019;45(1):5–12. doi:10.1111/jog.13782
39. Stadelmann P, Alessandri R, Eick S, Salvi GE, Surbek D, Sculean A. The potential association between gingival crevicular fluid inflammatory mediators and adverse pregnancy outcomes: A systematic review. *Clin Oral Investig*. 2013;17(6):1453–1463. doi:10.1007/s00784-013-0952-0
40. Puertas A, Magan-Fernandez A, Blanc V, et al. Association of periodontitis with preterm birth and low birth weight: A comprehensive review. *J Matern Fetal Neonatal Med*. 2018;31(5):597–602. doi:10.1080/14767058.2017.1293023
41. González-Jaranay M, Téllez L, Roa-López A, Gómez-Moreno G, Moreu G. Periodontal status during pregnancy and postpartum. *PLoS One*. 2017;12(5):e0178234. doi:10.1371/journal.pone.0178234
42. Radnai M, Gorzó I, Nagy E, Urbán E, Novák T, Pál A. A possible association between preterm birth and early periodontitis: Pilot study. *J Clin Periodontol*. 2004;31(9):736–741. doi:10.1111/j.1600-051X.2004.00564.x
43. Lindhe J, Socransky SS, Nyman S, Haffajee A, Westfelt E. "Critical probing depths" in periodontal therapy. *J Clin Periodonlol*. 1982;9(4):323–336. doi:10.1111/j.1600-051x.1982.tb02099.x
44. Nesse W, Abbas F, van Der Ploeg I, Lucien Spijkervet FK, Dijkstra PU, Vissink A. Periodontal inflamed surface area: Quantifying inflammatory burden. *J Clin Periodontol*. 2008;35(8):668–673. doi:10.1111/j.1600-051x.2008.01249.x
45. Beck JD, Offenbacher S. Relationships among clinical measures of periodontal disease and their associations with systemic markers. *Ann Periodontol*. 2002;7(1):79–89. doi:10.1902/annals.2002.7.1.79
46. López NJ, Smith PC, Gutierrez J. Higher risk of preterm birth and low birth weight in women with periodontal disease. *J Dent Res*. 2002;81(1):58–63. doi:10.1177/002203450208100113
47. Khader Y, Al-Shishani L, Obeidat B, et al. Maternal periodontal status and preterm low birth weight delivery: A case–control study. *Arch Gynecol Obstet*. 2009;279(2):165–169. doi:10.1007/s00404-008-0696-2
48. Gesase N, Miranda-Rius J, Brunet-Llobet L, Lahor-Soler E, Mahande MJ, Masenga G. The association between periodontal disease and adverse pregnancy outcomes in Northern Tanzania: A cross-sectional study. *Afr Health Sci*. 2018;18(3):601–611. doi:10.4314/ahs.v18i3.18
49. Zhang Y, Feng W, Li J, Cui L, Chen ZJ. Periodontal disease and adverse neonatal outcomes: A systematic review and meta-analysis. *Front Pediatr*. 2022;10:799740. doi:10.3389/fped.2022.799740
50. Manrique-Corredor EJ, Orozco-Beltran D, Lopez-Pineda A, Quesada JA, Gil-Guillen VF, Carratala-Munuera C. Maternal periodontitis and preterm birth: Systematic review and meta-analysis. *Community Dent Oral Epidemiol*. 2019;47(3):243–251. doi:10.1111/cdoe.12450

Original paper

Evaluation of the effects of different mouthrinses on dental remineralization

Selver Suna Basak[1,A–F], Eda Dokumacioglu[2,C–F]

[1] Department of Oral and Dental Health, Vocational School of Health Services, Artvin Çoruh University, Artvin, Turkey
[2] Department of Nutrition and Dietetics, Faculty of Health Sciences, Artvin Çoruh University, Artvin, 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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):219–225

Address for correspondence
Eda Dokumacioglu
E-mail: eda_ozcelik@artvin.edu.tr

Funding sources
The present work was supported by the Coordinator of Scientific Research Projects (2017.M80.02.01) at the Artvin Çoruh University, Artvin, Turkey.

Conflict of interest
None declared

Acknowledgements
None declared

Received on December 7, 2020
Reviewed on February 15, 2021
Accepted on March 15, 2021

Published online on June 16, 2023

Abstract

Background. Dental caries occurs with the release of organic acids from the fermentable carbohydrates metabolized by cariogenic microorganisms. Microbial, genetic, immunological, behavioral, and environmental factors play a role in the development and severity of dental caries.

Objectives. The aim of the present study was to investigate the possible effects of different mouthwash solutions on dental remineralization.

Material and methods. This in vitro study compared the remineralization capacity of different mouthwash solutions applied topically to the enamel surface. A total of 50 tooth specimens were prepared from the buccal and lingual halves, with 10 teeth in each group: G1 (control); G2 (Listerine®); G3 (Sensodyne®); G4 (Oral B® Pro-Expert); and G5 (DentaSave® Zinc). Remineralization capacity was evaluated in all groups. The one-way analysis of variance (ANOVA) and the paired samples *t* test were used for statistical analysis, with a p-value <0.05 considered significant.

Results. There were significant differences in the calcium (Ca)/phosphorus (P) atomic percentage (at%) ratio between the demineralized and remineralized dentin ($p = 0.001$), and between the demineralized and remineralized enamel ($p = 0.006$). Similarly, there were significant differences in the at% of P ($p = 0.017$) and zinc (Zn) ($p = 0.010$) between the demineralized and remineralized dentin. There was a significant difference in the at% of P ($p = 0.030$) between the demineralized and remineralized enamel. The Zn at% in enamel was significantly higher after remineralization in G5 as compared to the control group ($p < 0.05$). The images of the demineralized enamel showed the usual keyhole prism appearance, with intact prism sheaths and negligible inter-prism porosity.

Conclusions. The scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) findings seem to confirm the effectiveness of DentaSave Zinc for the remineralization of enamel lesions.

Keywords: tooth demineralization, X-ray emission, tooth remineralization, scanning electron microscopy

Cite as
Basak SS, Dokumacioglu E. Evaluation of the effects of different mouthrinses on dental remineralization. *Dent Med Probl.* 2023;60(2):219–225. doi:10.17219/dmp/134290

DOI
10.17219/dmp/134290

Introduction

Dental caries begins with the release of organic acids from the fermentable carbohydrates metabolized by cariogenic microorganisms. During this process, along with the imbalance between the tooth tissues and the plaque fluid, dental caries lesions occur as a result of the demineralization of the inorganic components of the tooth.[1] Microbial, genetic, immunological, behavioral, and environmental factors play a role in the development and severity of dental caries. Dental caries remains an important health problem today, although its prevalence has declined in many developed countries in respect to previous years.[2] Owing to the understanding of the pathological process of caries and the identification of the factors affecting the demineralization process, it is well established that initial caries lesions can be remineralized.[3]

If the environmental balance can be adjusted to promote remineralization when initial caries lesions occur, remineralization can be achieved by depositing the calcium (Ca) and phosphate ions lost from the enamel tissue back onto the enamel surface. The process requires eliminating the factors reducing intraoral pH, buffering the acids produced by certain bacteria by the saliva over time so that pH can increase to a neutral level, and the saturation of plaque and the saliva with the minerals dissolved in enamel.[4]

Remineralization is defined as the redeposition on the enamel surface of Ca, phosphorus (P) and other ions lost from the enamel tissue due to caries or other reasons; it is a process of enamel tissue regeneration.[5] The awareness of the importance of preventive oral and dental care is increasing day by day due to a better understanding of the multifactorial etiology underlying dental caries, the advanced technology of dental materials, and achievements in protective applications through early diagnosis methods and minimally invasive techniques.[6]

The present study aimed to investigate the possible dental remineralization effects of different mouthwash solutions available in Turkey.

Material and methods

This in vitro study was carried out at Artvin Oral and Dental Health Center and Artvin Çoruh University Central Research Laboratory, Turkey. Before commencing the study, the Non-Interventional Trials Ethics Committee at Artvin Çoruh University approved the protocol (No. 24/11/2017-E.20466).

Collection and preparation of dental specimens

In the 1st phase of the study, erupted upper and lower third molar samples were used; the teeth had no signs of caries, but they were extracted for various reasons within the previous month. After extraction, the teeth were kept in distilled water at +4°C until testing.[7] Soft tissue residues and additives were removed, and the teeth were cleansed using a fluorine-free pumice and a brush. To remove pumice remains, the teeth were washed for 15 s, left to dry, and then examined under a stereomicroscope. The ones determined to be free of caries, hypoplasia, fractures, and cracks were included in the study.

A total of 50 teeth were prepared from the buccal and lingual halves, with 10 teeth in each group: G1 (control; the remineralization solution); G2 (Listerine®; Lambert Pharmacal Company, St. Louis, USA); G3 (Sensodyne®; GlaxoSmithKline Consumer Healthcare, London, UK); G4 (Oral B® Pro-Expert; Procter & Gamble, Cincinnati, USA); and G5 (DentaSave® Zinc; Drogsan, Ankara, Turkey). Remineralization capacity was evaluated in all groups. To achieve this, the crowns of the teeth were removed from the roots with a 0.3-millimeter-thick, low-speed, high-precision, double-sided diamond separator. Then, the teeth were divided mesiodistally into 2 parts to obtain buccal and lingual surfaces, and ground using 600-grit, 800-grit, and 1,200-grit abrasive paper disks. The sanding direction was changed to remove any marks caused by the previous sanding application. The obtained enamel surfaces were examined under a stereomicroscope, and sanding was repeated in cases the surface was not smooth enough.[8]

The obtained samples were embedded in acrylic molds with an inner diameter of 15 mm and a height of 20 mm, with their upper and lower parts parallel to each other, and in such a way that the surfaces to be exposed to demineralization and remineralization were left open. Then, stickers were fixed in the middle of the samples to prepare 4 mm × 4 mm windows. The natural surfaces of the teeth outside the area concealed by the stickers were covered with acid-resistant varnish.[9]

Preparation of the artificial saliva solution

The artificial saliva solution was freshly prepared before the tests, using the composition described by Ten Cate et al.[10] The solution contained 1.28568 g NaCl, 0.0320 g $MgCl_2 \cdot 6H_2O$, 0.07945 g $CaCl_2 \cdot 2H_2O$, 0.29857 g KCl, 0.897 g KOH, and 472 µl H_3PO_4.

Preparation of the remineralization solution

The remineralization solution was freshly prepared before the tests according to the composition described by Ten Cate et al., with 1.0 mM $CaCl_2$, 2 mM KH_2PO_4 and 150 mM KCl.[10] It was preserved by adding 0.01% NaN_3, pH was adjusted to 7.0 by using 1M KOH, and it was kept at room temperature.

Preparation and application of the demineralization solution

The next phase involved inducing artificial caries lesions on the surfaces of 10 samples in each group. The samples were incubated for 7 days in capped containers, with 10 mL of the demineralization solution added to each sample. The demineralization solution was prepared by mixing 100 mmol/L NaOH and 100 mmol/L lactic acid (pH 5.0). To achieve a viscosity of 100 cp, 0.2 g/L carboxymethyl cellulose sodium salt was added to the solution.

Implementation of treatment procedures

The demineralized teeth were treated twice a day for 1 month, using different mouthwash solutions, as prescribed by each manufacturer.[11] Four different mouthwash solutions were applied to all samples at equal rates, with deionized water applied to the control group. The samples were stored in artificial saliva at 37°C for 7 days.

SEM imaging and EDS analysis

Scanning electron microscopy (SEM) imaging for assessing the mineral depositions caused by different materials applied to the enamel specimens with initial enamel lesions, as well as the energy-dispersive X-ray spectroscopy (EDS) analysis for determining changes in the mineral content, were performed using a SEM connected to an EDS detector (EVO LS 10; Carl Zeiss NTS, Oberkochen, Germany). For this purpose, the samples were coated with a 100-angstrom gold layer under a vacuum rate of 10^{-4} Tr, using a vacuum coating apparatus (EVO LS 10). After coating, the samples were placed on the SEM stage via aluminum tables, and SEM images were obtained at ×5,000 and ×2,000 magnifications at 20 kV and a working distance of 10 mm. After obtaining the images, the content of oxygen (O), P, Ca, and zinc (Zn) elements on the enamel surface were determined as percentages with the EDS detector.

Statistical analysis

All data is presented as mean (*M*) and standard deviation (*SD*). The Shapiro–Wilk test evaluated the normality of the data. The one-way analysis of variance (ANOVA) was applied to compare the mineral content among the 5 experimental groups. The mineral content of enamel and dentin before and after demineralization was compared with the paired samples *t* test. All analyses employed IBM SPSS Statistics for Windows, v. 19.0 (IBM Corp., Armonk, USA). A *p*-value <0.05 was considered statistically significant.

Results

The mineral content of each specimen in the 5 groups was measured by means of EDS. The chemical analysis of the demineralized enamel showed that it predominantly contained O, P, Ca, and Zn. The Ca/P atomic percentage (at%) ratio was approx. 1.78 for the demineralized enamel and 1.87 for the remineralized enamel (Table 1). The Ca/P at% ratio for the demineralized and remineralized dentin was 1.71 and 1.83, respectively (Table 2).

Table 1 shows the mean values for the enamel mineral content of 50 specimens before and after remineralization. There were no significant differences in the O (p = 0.292), Ca (p = 0.595) and Zn (p = 0.117) at%. There was a significant difference in the Ca/P at% ratio between the demineralized and remineralized enamel (p = 0.006). Similarly, there was a significant difference in the P at% between the demineralized and remineralized enamel (p = 0.030).

Table 2 shows the mean values for the dentin mineral content of 50 specimens before and after remineralization. There were no significant differences in the

Table 1. Enamel mineral content before and after remineralization

Element	Enamel	*n*	Mineral content	*p*-value
Oxygen (O) [at%]	demineralized	50	64.21 ±7.04	0.292
	remineralized	50	65.79 ±8.61	
Phosphorus (P) [at%]	demineralized	50	12.71 ±2.03	0.030*
	remineralized	50	11.80 ±2.69	
Calcium (Ca) [at%]	demineralized	50	22.76 ±4.70	0.595
	remineralized	50	22.21 ±5.98	
Zinc (Zn) [at%]	demineralized	50	0.12 ±0.08	0.117
	remineralized	50	0.19 ±0.28	
Ca/P at% ratio	demineralized	50	1.78 ±0.14	0.006*
	remineralized	50	1.87 ±0.16	

Data presented as mean ± standard deviation (*M* ±*SD*). * statistically significant ($p < 0.05$).

Table 2. Dentin mineral content before and after remineralization

Element	Dentin	*n*	Mineral content	*p*-value
Oxygen (O) [at%]	demineralized	50	69.48 ±5.76	0.462
	remineralized	50	70.49 ±8.28	
Phosphorus (P) [at%]	demineralized	50	11.11 ±1.98	0.017*
	remineralized	50	10.14 ±2.38	
Calcium (Ca) [at%]	demineralized	50	19.01 ±3.43	0.771
	remineralized	50	18.75 ±5.77	
Zinc (Zn) [at%]	demineralized	50	0.22 ±0.13	0.010*
	remineralized	50	0.62 ±1.06	
Ca/P at% ratio	demineralized	50	1.71 ±0.07	0.001*
	remineralized	50	1.83 ±0.21	

Data presented as *M* ±*SD*. * statistically significant ($p < 0.05$).

O (p = 0.462) and Ca (p = 0.771) at%. There was a significant difference in the Ca/P at% ratio between the demineralized and remineralized dentin (p = 0.001). Similarly, there were significant differences in the P (p = 0.017) and Zn (p = 0.010) at% between the demineralized and remineralized dentin. The Ca/P at% was slightly higher in enamel than in dentin.

The Zn at% in enamel was significantly higher after remineralization in G5 as compared to the control group ($p < 0.05$). However, there were no significant differences in the Zn levels between G2, G3 or G4 and the control group ($p > 0.05$). Also, there were no significant differences between the experimental groups and the control in terms of the O, P and Ca at%, and the Ca/P at% ratio ($p > 0.05$) (Table 3).

After remineralization, the Zn at% in dentin was significantly higher in G5 than in the control group ($p < 0.05$). In contrast, the Zn at% was significantly lower in G3 than in the control group ($p < 0.05$). The Zn at% was also lower in G2 and G4 than in the control group, but the differences were not statistically significant ($p > 0.05$). The O, P and Ca at% were not different between the groups or when compared to the control group ($p > 0.05$). As for the Ca/P at% ratio, G5 had a significantly higher ratio as compared to the control group ($p < 0.05$). The Ca/P at% ratio was also higher in G2 and G3 as compared to the control, but without statistical significance ($p > 0.05$). Meanwhile, the Ca/P at% ratio in G4 was lower than in the control, but again without statistical significance ($p > 0.05$) (Table 4).

Figures 1–5 show representative SEM images (×2,000 magnification) of dentin after remineralization for different treatment groups. The specimens from the 5 treatment groups showed some dentin remineralization on the top surface, with a more homogeneous and denser mineral content. Also, the surfaces of the control group samples were densely covered with the mineral content. The specimens treated with Oral B Pro-Expert and DentaSave Zinc had a surface layer of irregular and porous minerals deposited. The groups treated with Listerine and Sensodyne had a remineralized layer with a more homogeneous and denser dentin mineral content that went deeper into the demineralized region.

Table 3. Effect of mouthwash solutions on the enamel mineral content after remineralization

Element	G1	G2	G3	G4	G5
Oxygen (O) [at%]	71.74 ±6.63	64.51 ±9.40	64.43 ±10.77	65.88 ±7.04	62.38 ±7.02
Phosphorus (P) [at%]	9.99 ±2.39	11.86 ±2.96	12.39 ±3.11	12.08 ±2.28	12.67 ±2.28
Calcium (Ca) [at%]	18.19 ±4.25	23.51 ±6.61	23.11 ±7.78	21.91 ±4.74	24.34 ±4.90
Zinc (Zn) [at%]	0.08 ±0.09	0.04 ±0.05	0.08 ±0.51	0.13 ±0.12	0.61 ±0.39[a]
Ca/P at% ratio	1.85 ±0.08	1.98 ±0.19	1.84 ±0.20	1.81 ±0.11	1.92 ±1.17

Data presented as *M* ±*SD*. Groups: G1 – control; G2 – Listerine; G3 – Sensodyne; G4 – Oral B Pro-Expert; and G5 – DentaSave Zinc. [a] statistically significantly different when compared to the control group ($p < 0.05$).

Table 4. Effect of mouthwash solutions on the dentin mineral content after remineralization

Element	G1	G2	G3	G4	G5
Oxygen (O) [at%]	75.85 ±5.84	67.62 ±8.42	67.28 ±8.53	75.35 ±6.57	66.36 ±7.36
Phosphorus (P) [at%]	8.67 ±2.33	11.15 ±2.37	11.27 ±1.94	9.35 ±2.46	10.24 ±2.05
Calcium (Ca) [at%]	15.32 ±3.49	21.11 ±6.12	21.38 ±6.67	15.16 ±4.06	20.76 ±4.96
Zinc (Zn) [at%]	0.18 ±0.07	0.10 ±0.10	0.07 ±0.08[a]	0.14 ±0.12	2.64 ±0.65[a]
Ca/P at% ratio	1.78 ±0.12	1.88 ±0.15	1.87 ±0.28	1.62 ±0.10	2.01 ±0.17[a]

Data presented as *M* ±*SD*. Groups: G1 – control; G2 – Listerine; G3 – Sensodyne; G4 – Oral B Pro-Expert; and G5 – DentaSave Zinc. [a] statistically significantly different when compared to the control group ($p < 0.05$).

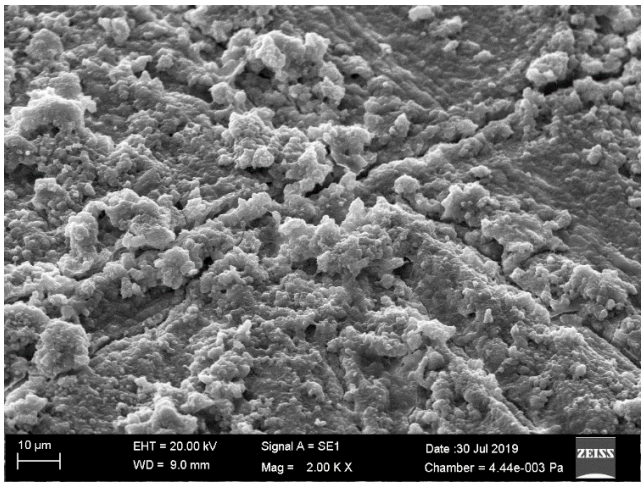

Fig. 1. Scanning electron microscopy (SEM) image of the remineralized dentin at ×2,000 magnification in G1

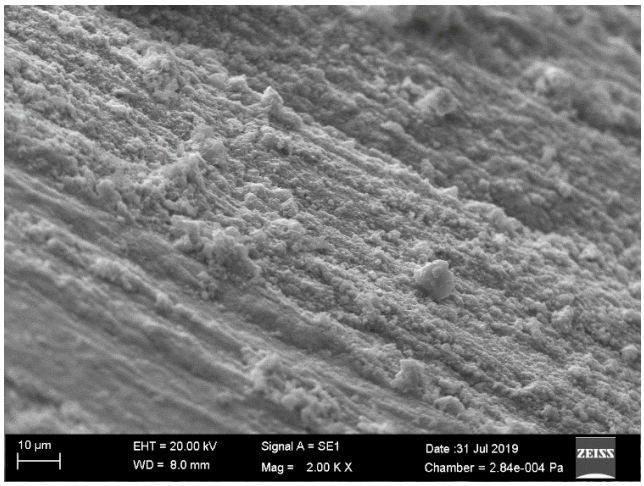

Fig. 2. Scanning electron microscopy (SEM) image of the remineralized dentin at ×2,000 magnification in G2

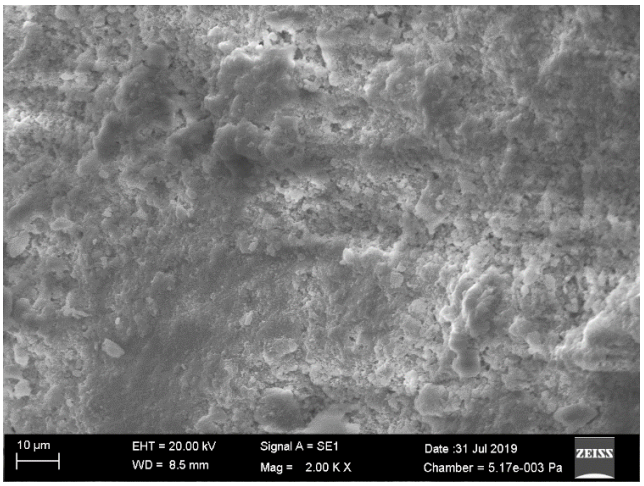

Fig. 3. Scanning electron microscopy (SEM) image of the remineralized dentin at ×2,000 magnification in G3

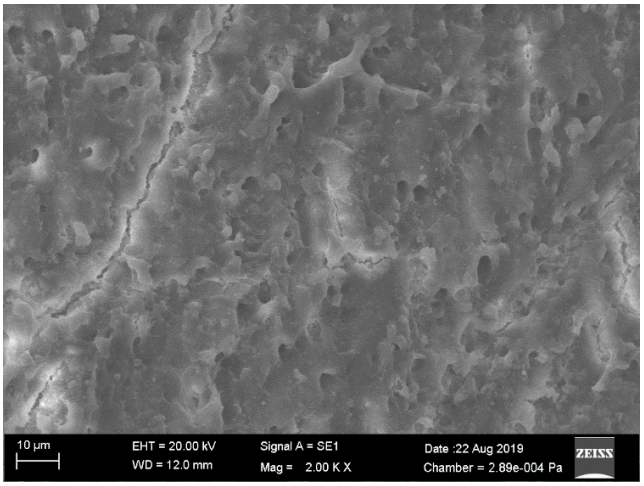

Fig. 4. Scanning electron microscopy (SEM) image of the remineralized dentin at ×2,000 magnification in G4

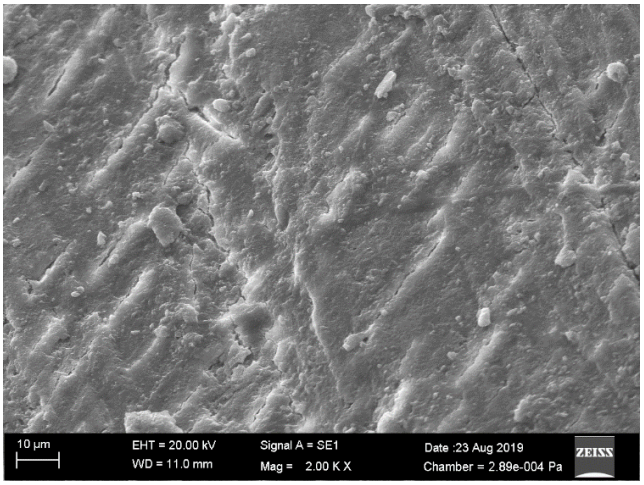

Fig. 5. Scanning electron microscopy (SEM) image of the remineralized dentin at ×2,000 magnification in G5

Discussion

As a significant health problem, dental caries is a pathological process characterized by the localized destruction of dental hard tissues by cariogenic microorganisms.[12] Dental hard tissues are constantly influenced by intraoral pH changes, and a process called demineralization occurs when pH is below 5.5. During demineralization, Ca and phosphate ions are dissolved and released from the enamel structure, causing enamel loss.[13,14] The dissolved ions accumulate on the enamel surface with the raised pH levels, which initiates the remineralization process.[15] Various products and foods with a low acid content cause the intraoral pH level to increase. In recent years, a wide variety of products preventing demineralization have become available. Therefore, the present study aimed to determine the remineralization capacity of 4 products of different content.

It is known that various processes and different materials applied to the surfaces of dental hard tissues can change their mineral composition and structure. Hydroxyapatite crystal is the main inorganic component of enamel, and the size, permeability and solubility of the crystal are significantly affected by changes in the amount of Ca and P with respect to other elements that constitute its structure.[16] The Ca and P at%, as well as the Ca/P at% ratio, are higher in intact enamel than in the demineralized tooth tissue. For this reason, studies on the remineralization of dental hard tissues generally evaluate Ca and P elements and the Ca/P ratio.[17,18] In the current study, we determined the Ca and P at% and the Ca/P at% ratio in both enamel and dentin. In enamel, the Ca/P at% ratio showed a significant increase after remineralization as compared to the condition after demineralization. Meanwhile, the P at% showed a significant decrease after remineralization as compared to the level during the demineralization process. The Ca level also decreased after remineralization, but the change was not statistically significant. We also evaluated the O and Zn at% in both enamel and dentin. There were no statistically significant differences in the O and Zn at% after enamel remineralization as compared to the values after enamel demineralization. As for the O, P, Ca, and Zn at% and the Ca/P at% ratio in dentin, the Zn level and the Ca/P at% ratio increased significantly after remineralization as compared to demineralization, whereas the P level decreased. The process of remineralization led to a reduction in the Ca and P losses occurring in both enamel and dentin.

Mouthwashes are non-sterile aqueous solutions with a fragrant, refreshing and antiseptic effect. Mouthwashes are used in dentistry as protective and therapeutic agents, and are helpful during some professional procedures. With different content and forms, as well as due to the ease of use, they constitute a vital aspect of preventive treatment for physicians and patients. They are designed to reduce the count of oral bacteria, remove residual food particles, temporarily eliminate bad breath, and leave a pleasant taste in the mouth.[19,20] Dietary acids are the crucial external etiologic factors of dental erosion. Effective agents that can prevent and treat dental erosion

should be investigated. For this reason, the present study evaluated the fluorine (F)-containing Listerine, Sensodyne and Oral B Pro-Expert, and Zn-containing DentaSave Zinc products.

For caries prevention, the World Health Organization (WHO) recommends using 0.05% sodium fluoride mouthwash (230 ppm F) daily, or 0.2% sodium fluoride mouthwash (900 ppm F) once a week or every 15 days.[21] In the literature, fluoride mouthwashes were reported to be effective in preventing caries at a rate of 26%.[22,23] In the current study, DentaSave Zinc displayed a better performance in preventing enamel and dentin erosion, as well as in the remineralization of the enamel–dentin surface, as compared to the other mouthwash products and the control group. Indeed, the Ca/P at% ratio and the Zn at% were significantly higher in the DentaSave Zinc group than in the control group. Furthermore, there were no statistically significant differences between the Listerine, Sensodyne and Oral B Pro-Expert applications and the control group concerning the O, P and Ca at% and the Ca/P at% ratio.

Initial enamel lesions have the potential to progress very rapidly, and there is a balance between the demineralization and remineralization cycles in the oral environment. The main goal is shifting the mineral balance in the mouth in favor of tooth remineralization, with various products used for this process. Dental cavitation may occur if no preventive measures are taken against the progression of enamel lesions.[24] Erosion was determined on the enamel surfaces of all specimens in a pH-cycling model mimicking the oral environment. The SEM analysis showed the morphological changes caused by treating the induced enamel and dentin lesions with various products. The EDS analysis showed increased enamel and dentin Ca/P at% ratio following the treatment. The above findings seem to converge and agree on the effectiveness of DentaSave Zinc for the remineralization of enamel lesions.

Conclusions

This study was limited to the evaluated mouthwashes. Also, the remineralization effects of these mouthwashes were studied only in enamel and dentin. Although the chemistry of the demineralization–remineralization process is similar in dentin and root cement, the structure of each of these materials, and their mineral and organic tissue content are different, which causes differences in the formation and progression of the carious lesion. The applications of the Listerine, Sensodyne or Oral B Pro-Expert mouthwashes was not as effective in terms of tooth remineralization as DentaSave Zinc. Nonetheless, the remineralization capacity of these mouthwashes in dentin should also be investigated. Therefore, further clinical studies should be conducted.

Ethics approval and consent to participate

The study was approved by the Non-Interventional Trials Ethics Committee at Artvin Çoruh University, Artvin, Turkey (No. 24/11/2017-E.20466).

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

Selver Suna Basak https://orcid.org/0000-0003-1373-9579
Eda Dokumacioglu https://orcid.org/0000-0002-2223-1331

References

1. Goswami M, Saha S, Chaitra TR. Latest developments in non-fluoridated remineralizing technologies. *J Indian Soc Pedod Prev Dent*. 2012;30(1):2–6. doi:10.4103/0970-4388.95561
2. Murdoch-Kinch CA, McLean ME. Minimally invasive dentistry. *J Am Dent Assoc*. 2003;134(1):87–95. doi:10.14219/jada.archive.2003.0021
3. Songsiripradubboon S, Hamba H, Trairatvorakul C, Tagami J. Sodium fluoride mouthrinse used twice daily increased incipient caries lesion remineralization in an in situ model. *J Dent*. 2014;42(3):271–278. doi:10.1016/j.jdent.2013.12.012
4. Abou Neel EA, Aljabo A, Strange A, et al. Demineralization–remineralization dynamics in teeth and bone. *Int J Nanomedicine*. 2016;11:4743–4763. doi:10.2147/IJN.S107624
5. Jones RS, Darling CL, Featherstone JD, Fried D. Remineralization of in vitro dental caries assessed with polarization-sensitive optical coherence tomography. *J Biomed Opt*. 2006;11(1):014016. doi:10.1117/1.2161192
6. Elderton RJ. Preventive (evidence-based) approach to quality general dental care. *Med Princ Pract*. 2003;12(Suppl 1):12–21. doi:10.1159/000069841
7. Eloy Dantas DC, Meira Ribeiro AI, Marques de Almeida Lima LH, et al. Influence of water storage time on the bond strength of etch-and-rinse and self-etching adhesive systems. *Braz Dent J*. 2008;19(3):219–223. doi:10.1590/s0103-64402008000300008
8. Yilmaz N, Ocak M, Ökte Z. Remineralization of primary molar dentine with silver diamine fluoride and sodium fluoride: An in vitro study. *Cumhuriyet Dent J*. 2020;23(4):340–347. doi:10.7126/cumudj.796823
9. Gonenc O, Sandalli N. Comparing tooth surface microhardness with mineral density levels in a newly developed remineralisation agents when applied on artificial caries lesions (published PhD dissertation). Istanbul, Turkey: Yeditepe University; 2015.
10. Ten Cate JM, Larsen MJ, Pearce EI, Fejerskov O. Chemical interactions between the tooth and oral fluids. In: Fejerskov O, Kidd EA, eds. *Dental Caries: The Disease and its Clinical Management*. 8th ed. Oxford, UK: Blackwell Publishing; 2003:46–69.
11. Kawasaki K, Kambara M. Effects of ion-releasing tooth-coating material on demineralization of bovine tooth enamel. *Int J Dent*. 2014;2014:463149. doi:10.1155/2014/463149
12. Chen F, Wang D. Novel technologies for the prevention and treatment of dental caries: A patent survey. *Expert Opin Ther Pat*. 2010;20(5):681–694. doi:10.1517/13543771003720491
13. Kucukyilmaz E, Savas S. Measuring the remineralization potential of different agents with quantitative light-induced fluorescence digital Biluminator. *J Appl Biomater Funct Mater*. 2017;15(1):e101–e106. doi:10.5301/jabfm.5000317

14. Dawes C. What is the critical pH and why does a tooth dissolve in acid? *J Can Dent Assoc*. 2003;69(11):722–724. PMID:14653937.
15. Featherstone JD. Dental caries: A dynamic disease process. *Aust Dent J*. 2008;53(3):286–291. doi:10.1111/j.1834-7819.2008.00064.x
16. Contreras-Bulnes R, Olea-Mejía OF, Rodríguez-Vilchis LE, Scougall-Vilchis RJ, Centeno-Pedraza C. Structural changes on human dental enamel treated with Er:YAG, CO_2 lasers and remineralizing solution: EDS analysis. In: Virdi M, ed. *Oral Health Care - Prosthodontics, Periodontology, Biology, Research and Systemic Conditions*. InTech. 2012:299–318. doi:10.5772/28867
17. Amaechi BT, Porteous N, Ramalingam K, et al. Remineralization of artificial enamel lesions by theobromine. *Caries Res*. 2013;47(5):399–405. doi:10.1159/000348589
18. Yang JH, Oh KJ, Pandher DS. Hydroxyapatite crystal deposition causing rapidly destructive arthropathy of the hip joint. *Indian J Orthop*. 2011;45(6):569–572. doi:10.4103/0019-5413.87139
19. Aytaç F, Erklı H, Koser C, Ersöz E. Assessment of remineralization potential of CPP-ACP [In Turkish]. *Turkiye Klinikleri J Dental Sci*. 2012;18(3):258–263.
20. Zor ZF, Çevik P. Removal of dental stains induced by antibacterial mouthwashes by using different bleaching techniques [in Turkish]. *Selcuk Dent J*. 2018;5:218–224. doi:10.15311/selcukdentj.311303
21. McDonald RE, Avery DR, George KS. Dental caries in the child and adolescent. In: McDonald RE, Avery DR, Dean JA, eds. *McDonald and Avery's Dentistry for the Child and Adolescent*. 8th ed. St. Louis, MO: Mosby; 2004:205–232.
22. Sharma A, Agarwal N, Anand A, Jabin Z. To compare the effectiveness of different mouthrinses on *Streptococcus mutans* count in caries active children. *J Oral Biol Craniofac Res*. 2018;8(2):113–117. doi:10.1016/j.jobcr.2018.05.002
23. Bağış YH, Bağış N. Investigation of antibacterial effects of different mouth rinses: An in-vitro study [in Turkish]. *J Dent Fac Ataturk Uni*. 2019;46(1):1–6.
24. Bostancı B, Korkut E, Ünlü N. Non-fluoridated and non-invasive treatment methods of initial enamel lesions. *Turkiye Klinikleri J Restor Dent-Special Topics*. 2017;3(1):7–13.

Original paper

Oral health-related quality of life and xerostomia in type 2 diabetic patients

Adel Tabesh[1,A–F], Mahboobeh Mahmood[2,A–F], Samin Sirous[3,A–F]

[1] Department of Oral Medicine, Dental Research Center, Dental Research Institute, Faculty of Dentistry, Isfahan University of Medical Sciences, Iran
[2] Department of Restorative Dentistry, School of Dentistry, Tehran University of Medical Sciences, Iran
[3] Department of Periodontics, UCLA School of Dentistry, Los Angeles, USA

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):227–231

Address for correspondence
Mahboobeh Mahmood
E-mail: mahbobeh.md@gmail.com

Funding sources
The study was financially supported by Isfahan University of Medical Sciences, Iran (grant No. 399551).

Conflict of interest
None declared

Acknowledgements
None declared

Received on December 18, 2021
Reviewed on March 20, 2022
Accepted on March 28, 2022

Published online on June 27, 2023

Abstract

Background. Diabetes mellitus (DM) is a known risk factor for xerostomia. Oral health-related quality of life (OHRQoL) is a multi-dimensional issue reflecting several effects of the oral condition on the quality of life.

Objectives. The present study aimed to assess OHRQoL and its relationship with xerostomia severity in type 2 diabetic patients.

Material and methods. A total of 200 patients participated in this cross-sectional study. The Xerostomia Inventory (XI) assessed xerostomia severity and the Oral Health Impact Profile-14 (OHIP-14) questionnaire evaluated OHRQoL. In addition, the fasting blood sugar (FBS) and glycated hemoglobin (HbA1c) tests were conducted, and the results were recorded, as well as the disease duration and denture wearing. Data analysis employed the *t* test and Pearson's correlation coefficient.

Results. The mean XI score was 22.27 ±6.92 and the mean OHIP-14 score was 13.76 ±8.41. The mean FBS, HbA1c and disease duration values were 161.23 ±49.14 mg/dL, 7.90 ±1.12% and 11.02 ±7.78 years, respectively. The OHIP-14 score correlated significantly with the XI score, age, FBS, HbA1c, the disease duration, and denture wearing ($p < 0.05$).

Conclusions. There was a significant correlation between OHRQoL and xerostomia severity in patients with type 2 DM. Age, denture wearing, the disease duration, and the medical management of DM also correlated significantly with OHRQoL. Treating both the underlying disease and oral health comorbidities, such as xerostomia, seems to be essential for achieving a better OHRQoL in type 2 diabetic patients.

Keywords: xerostomia, diabetes, oral health, quality of life

Cite as
Tabesh A, Mahmood M, Sirous S. Oral health-related quality of life and xerostomia in type 2 diabetic patients. *Dent Med Probl.* 2023;60(2):227–231. doi:10.17219/dmp/147754

DOI
10.17219/dmp/147754

Introduction

Diabetes mellitus (DM) is a metabolic disorder typically characterized by the triad of polyphagia, polydipsia and polyuria.[1] Most DM patients are elderly with type 2 disease.[2] Amongst miscellaneous side effects, oral health is severely affected by DM as a result of hyperglycemia, impaired healing, and qualitative or quantitative salivary alterations.[3,4] Caries, oral burning, malodor, and periodontal problems are the common comorbidities of xerostomia in these patients, and might influence their oral health-related quality of life (OHRQoL).[5]

Xerostomia is a subjective feeling of a dry mouth, and it can be caused by many local or systemic factors, including direct damage to salivary tissue.[6] Several medical conditions may precede or exacerbate xerostomia, such as Sjögren's syndrome, systemic lupus erythematosus and sarcoidosis, as well as metabolic diseases, like DM.[7] Polyuria, dehydration and autonomic imbalance due to angiopathic disturbances have been proposed to underlie xerostomia in DM.[8] Regardless of its cause, xerostomia has extraordinarily detrimental effects on oral health. Rampant caries, periodontitis, and a reduced ability to chew or speak are significant side effects that can clearly influence a patient's OHRQoL.[9]

The OHRQoL score reflects the impact of the oral health status on several aspects of one's daily life. Oral health-related quality of life is measured with the use of patient-centered approaches. The OHRQoL score combined with clinical criteria constitute a suitable technique for evaluating oral treatment needs and outcomes.[10] Means available to achieve such a goal are reliable questionnaires, validated for specific populations. The Oral Health Impact Profile-14 (OHIP-14) questionnaire is a popular tool in this regard, and it is applied in xerostomic and DM patients.[2] Some studies have utilized this questionnaire to assess OHRQoL in DM and other systemic conditions.[2–6,10–12]

Molania et al. concluded that low medical control of type 2 DM resulted in hyposalivation as a side effect of the disease, and xerostomia affected the OHRQoL of DM patients in a negative way.[6] Xerostomia is a significant oral side effect of DM that may interfere with the oral function of the patients suffering from the disease, resulting in poor OHRQoL among them.[2] Therefore, evaluating the relationship between xerostomia and OHRQoL in people with DM might help clinicians to prioritize the treatment planned for DM patients. The present study investigated OHRQoL and its relationship with xerostomia severity in type 2 DM patients. The null hypothesis of the present investigation was that there is no correlation between OHRQoL and xerostomia severity in type 2 DM patients.

Material and methods

The present analytical cross-sectional study was conducted between September 2020 and February 2021.

Ethical considerations

The local medical ethics committee at Isfahan University of Medical Sciences, Iran, approved the study protocol (IRI.MUI.RESEARCH.REC.1399.505). The patients were informed of the objectives of the investigation, the confidentiality of the data, and that they could stop participating in the study at any time. They all provided written consent, and dental treatment was provided to them irrespective of whether they participated in the study or not.

Participants

Type 2 diabetic patients referred to the Department of Oral Medicine at the School of Dentistry of Isfahan University of Medical Sciences, Iran, were invited to participate in the present study.

Patients who met the following criteria were included: a confirmed diagnosis of type 2 DM; and literacy sufficient to fill out the questionnaires.

The exclusion criteria to minimize bias were the presence of any systemic diseases affecting the salivary glands, such as Sjögren's syndrome, alcoholism, a corticosteroid or hormone therapy, and a history of head and neck radiotherapy/chemotherapy (Fig. 1).

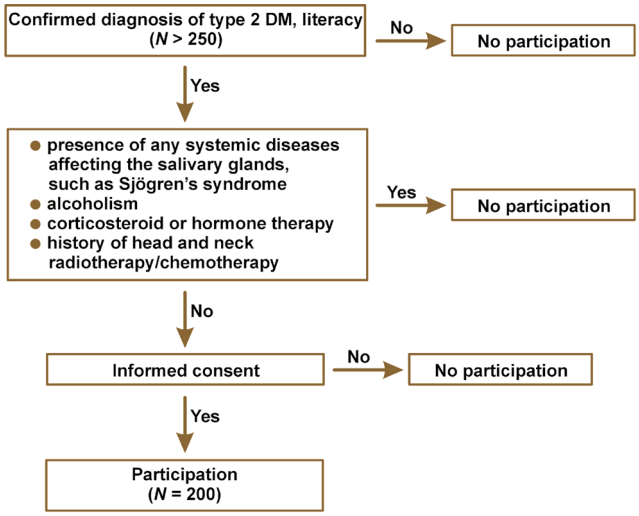

Fig. 1. Patient selection flowchart

DM – diabetes mellitus.

Sample size calculation

The following formula (Equation 1) was used to calculate the study sample size:

$$N = \frac{(z_{1-\alpha/2} + z_{1-\beta})^2}{d^2} + 3 \quad (1)$$

Assuming $\alpha = 0.05$ (significance level) and $1-\beta = 0.8$ (power), $z_{1-\alpha/2}$ and $z_{1-\beta}$ were considered 1.96 and 0.84, respectively. The correlation analyses used $d = 0.2$. The calculation indicated $N = 199$, and $N = 200$ was set for the sample size.

Xerostomia evaluation

To measure xerostomia severity, the participants were asked to answer the questions in the Xerostomia Inventory (XI) (Table 1). The XI is composed of 11 questions regarding a dry mouth feeling, and the score for each question varies on a Likert scale from 1 to 5, with worse conditions scoring more, as follows: never – 1; seldom – 2; sometimes – 3; often – 4; and always – 5. Therefore, xerostomia severity was reported as a sum between 11 and 55. The Persian version of the XI questionnaire was used, which was valid and reliable.[13]

Table 1. Xerostomia Inventory (XI)

No.	Statement
1	I sip liquids to help swallow food
2	My mouth feels dry when eating a meal
3	I get up at night to drink
4	My mouth feels dry
5	I have difficulty eating dry foods
6	I suck sweets or cough lozenges to relieve dry mouth
7	I have difficulty swallowing certain foods
8	The skin of my face feels dry
9	My eyes feel dry
10	My lips feel dry
11	The inside of my nose feels dry

OHRQoL evaluation

Each patient was then asked to fill out the OHIP-14 questionnaire, which consists of 14 questions measuring OHRQoL in 7 domains. The score for each question varies on a Likert scale from 0 (never) to 4 (always). As a result, the score for each section is a sum between 0 and 8, and the total score of the questionnaire ranges from 0 to 56. A higher score in this questionnaire indicates a lower OHRQoL. The Persian version of the OHIP-14 questionnaire was used, which was valid and reliable.[14]

Data collection

The patients' medical and dental records, as well as denture wearing, the duration of the disease, and the latest fasting blood sugar (FBS) and glycated hemoglobin (HbA1c) test values were recorded. Demographic data regarding patients' age and gender were also recorded and attached to the questionnaires.

Statistical analysis

The data was analyzed with IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA), using relevant statistical tests, with the *t* test used to compare the OHIP-14 and XI scores between the gender groups. Pearson's correlation coefficient assessed the relationship between the OHIP-14 and XI scores. The level of significance was set at $p < 0.05$.

Results

Among more than 250 patients referred during the study period, 200 who fulfilled the eligibility criteria and gave informed consent participated in the study (Fig. 1). The mean age of the participants was 62.42 ±10.04 years, with 63.5% being female and 36.5% male. The mean FBS and HbA1c values were 161.23 ±49.14 mg/dL and 7.90 ±1.12%, respectively. The mean duration of the disease was 11.02 ±7.78 years. Denture (complete or removable partial) wearers composed 45% of the sample. There was no missing data.

The mean XI score was 22.27 ±6.92, and the total and domain OHIP-14 scores are shown in Table 2. There was a direct and statistically significant relationship between the XI and OHIP-14 total/domain scores (Table 2). The relationship between the OHIP-14 score and other study variables is shown in Table 3. Figure 2 shows that as the XI score increased, the total OHIP-14 score also increased, which translates into worse OHRQoL ($p < 0.001$; $r = 0.444$).

Table 2. Oral Health Impact Profile-14 (OHIP-14) questionnaire total and domain scores related to the Xerostomia Inventory (XI) score

OHIP-14 domain	Score *M* ±*SD*	*p*-value	Pearson's *r*
Functional limitation	2.15 ±1.59	<0.001*	0.503
Physical pain	2.46 ±1.89	0.001*	0.247
Psychological discomfort	2.35 ±1.84	<0.001*	0.275
Physical disability	1.91 ±1.60	0.003*	0.230
Psychological disability	1.83 ±1.68	<0.001*	0.292
Social disability	1.39 ±1.51	<0.001*	0.332
Handicap	1.65 ±1.55	<0.001*	0.383
Total	13.76 ±8.41	<0.001*	0.444

M – mean; *SD* – standard deviation; *r* – Pearson's correlation coefficient; * statistically significant.

Table 3. Relationship between the Oral Health Impact Profile-14 (OHIP-14) score and other study variables

Correlation	Study variable	*p*-value	Pearson's *r*
OHIP-14 score	age	<0.001*	0.254
	gender	0.815	–
	FBS	0.040*	0.146
	HbA1c	0.030*	0.198
	disease duration	<0.001*	0.421
	denture wearing	<0.001*	–

FBS – fasting blood sugar; HbA1c – glycated hemoglobin; * statistically significant.

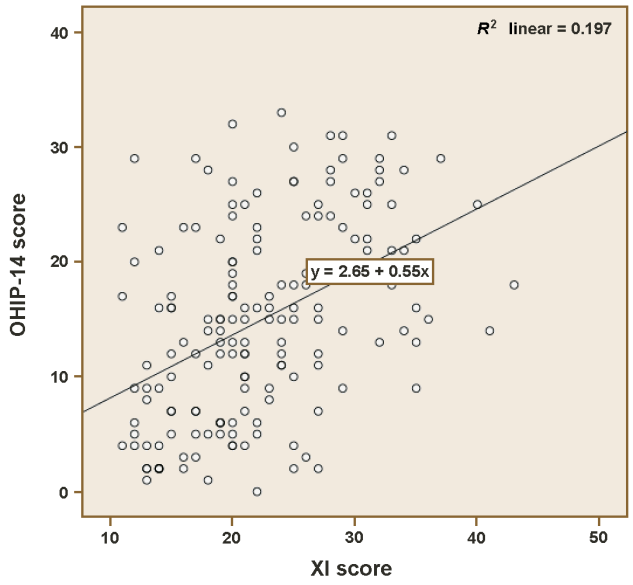

Fig. 2. Relationship between the Oral Health Impact Profile-14 (OHIP-14) score and the Xerostomia Inventory (XI) score ($p < 0.001$; $r = 0.444$)

Discussion

Xerostomia is a side effect of type 2 DM that can have detrimental consequences on a patient's oral health.[2] The present study was conducted to evaluate OHRQoL in patients with type 2 DM and its correlation with xerostomia severity. The mean OHIP-14 score was 13.86 ±8.41, which was relatively low, depicting a good OHRQoL. Moreover, a significant correlation was observed between the above-mentioned variables.

Several studies have investigated OHRQoL in type 2 DM or other medically compromised patients. Similar to our results, Sadeghi et al. reported acceptable OHRQoL in Persian diabetics,[15] and Hajian-Tilaki et al. reported a relatively good OHRQoL in Persian hemodialysis patients.[11] Machado et al.,[12] Pereira Oliveira et al.[10] and Verhulst et al.[3] came across even lower OHIP-14 scores in diabetic patients in comparison with our study, reporting mean scores of 9.5 ±11.3, 5.37 ±4.95 and 2.5 ±5.2, respectively. Of note, Hsu et al.[4] and Verhulst et al.[3] found lower OHIP-14 domain scores as compared to our study.

On the other hand, Khalifa et al.[5] and Irani et al.[16] reported no difference in the OHIP-14 scores in people with DM vs. healthy controls. Meanwhile, Mohamed et al. reported worse OHRQoL in Sudanese diabetic patients in comparison with the matched controls,[17] and Molania et al. reported a higher OHIP-14 score than our results.[6] Geographical variances and miscellaneous understandings of OHRQoL might explain the differences in the overall health support given to patients in different countries and even cities in the same country, which may be in line with their socioeconomic status.

The OHIP-14 questionnaire has proven to be a valuable tool for the subjective measurement of oral health in DM.[2] The present study showed a significant relationship between the OHIP-14 score and self-perceived xerostomia severity in diabetic patients, in line with studies by Nikbin et al.,[2] Molania et al.[6] and Azogui-Lévy et al.[18] These findings confirm its value and adaptation to other means of examining the clinical oral status. In fact, combining the subjective means of need evaluation with the classic objective methods provides patients with the best remedies for improving their OHRQoL.[12]

Oral health-related quality of life refers to both general and oral aspects of health.[2,3] In the present study, the indices related to the underlying disease (i.e., FBS, HbA1c, the disease duration, and age) and oral health conditions (i.e., the XI score and denture wearing) were significantly correlated with the OHIP-14 score. In line with our results, Sadeghi et al. found direct correlations between the OHIP-14 score and age and the disease duration in diabetic patients.[15]

On the contrary, Machado et al.[12] and Azogui-Lévy et al.[18] reported that the elderly experienced better OHRQoL among people with DM. Meanwhile, Irani et al. concluded that the burden of medical conditions in diabetic patients (e.g., multiple drug consumption) deteriorates OHRQoL so heavily that oral health finds no room to show its impact; therefore, the underlying medical condition seems to be a better predictor of OHRQoL than the oral health indices in diabetic patients.[16]

From another point of view, several studies have highlighted the impact of oral health on OHRQoL in diabetics, especially regarding its physical domains. The present study found the most significant impact on the 'physical pain' domain, and the strongest correlation with the XI score was found for the 'functional limitation' domain ($p < 0.001$; $r = 0.503$). The most important oral health parameters noted in the literature include denture wearing and xerostomia, similar to our findings.[1,2,4–6,10,19]

Several studies have also reported a strong correlation between xerostomia and OHRQoL, especially its physical domains, in the general population.[7,20–23] Since various factors potentially impact OHRQoL, including general and oral health parameters, planning treatment models to simultaneously improve general and oral health, as proposed by Machado et al.,[12] seems necessary for OHRQoL improvement in diabetic patients.

Limitations

Of course, this investigation was conducted within the limitations of a cross-sectional study; therefore, detecting the exact effect of xerostomia on OHRQoL might have been confounded by other variables with an impact on OHRQoL. Future research is suggested, with case–control or other controlled studies, to more precisely investigate the impact of xerostomia or other specific oral health parameters on OHRQoL among diabetic patients in order to improve their quality of life.

Conclusions

There was a significant correlation between OHRQoL and xerostomia severity in patients with type 2 DM. Furthermore, age, denture wearing, the disease duration, and the medical management of DM were other factors influencing OHRQoL in these patients. Prompt medical treatment of the underlying disease, as well as alleviating xerostomia, seem to be essential factors in improving OHRQoL in type 2 diabetic patients.

Ethics approval and consent to participate

The study was approved by the local medical ethics committee at Isfahan University of Medical Sciences, Iran (IRI.MUI.RESEARCH.REC.1399.505). All the participants provided informed written consent.

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

Adel Tabesh https://orcid.org/0000-0002-2012-8576
Mahboobeh Mahmood https://orcid.org/0000-0001-6603-3292
Samin Sirous https://orcid.org/0000-0002-5535-9083

References

1. Cervino G, Terranova A, Briguglio F, et al. Diabetes: Oral health related quality of life and oral alterations. *Biomed Res Int*. 2019;2019:5907195. doi:10.1155/2019/5907195
2. Nikbin A, Bayani M, Jenabian N, Khafri S, Motallebnejad M. Oral health-related quality of life in diabetic patients: Comparison of the Persian version of Geriatric Oral Health Assessment Index and Oral Health Impact Profile: A descriptive-analytic study. *J Diabetes Metab Disord*. 2014;13(1):32. doi:10.1186/2251-6581-13-32
3. Verhulst MJ, Teeuw WJ, Gerdes VE, Loos BG. Self-reported oral health and quality of life in patients with type 2 diabetes mellitus in primary care: A multi-center cross-sectional study. *Diabetes Metab Syndr Obes*. 2019;12:883–899. doi:10.2147/DMSO.S207087
4. Hsu YJ, Lin KD, Chen JH, et al. Periodontal treatment experience associated with oral health-related quality of life in patients with poor glycemic control in type 2 diabetes: A case–control study. *Int J Environ Res Public Health*. 2019;16(20):4011. doi:10.3390/ijerph16204011
5. Khalifa N, Rahman B, Gaintantzopoulou MD, Al-Amad S, Awad MM. Oral health status and oral health-related quality of life among patients with type 2 diabetes mellitus in the United Arab Emirates: A matched case–control study. *Health Qual Life Outcomes*. 2020;18(1):182. doi:10.1186/s12955-020-01418-9
6. Molania T, Alimohammadi M, Akha O, Mousavi J, Razvini R, Salehi M. The effect of xerostomia and hyposalivation on the quality of life of patients with type II diabetes mellitus. *Electron Physician*. 2017;9(11):5814–5819. doi:10.19082/5814
7. Niklander S, Veas L, Barrera C, Fuentes F, Chiappini G, Marshall M. Risk factors, hyposalivation and impact of xerostomia on oral health-related quality of life. *Braz Oral Res*. 2017;31:e14. doi:10.1590/1807-3107BOR-2017.vol31.0014
8. Mortazavi H, Baharvand M, Movahhedian A, Mohammadi M, Khodadoustan A. Xerostomia due to systemic disease: A review of 20 conditions and mechanisms. *Ann Med Health Sci Res*. 2014;4(4):503–510. doi:10.4103/2141-9248.139284
9. Van de Rijt LJ, Stoop CC, Weijenberg RA, et al. The influence of oral health factors on the quality of life in older people: A systematic review. *Gerontologist*. 2020;60(5):e378–e394. doi:10.1093/geront/gnz105
10. Pereira Oliveira EJ, Brasil Rocha VF, Nogueira DA, Pereira AA. Quality of life and oral health among hypertensive and diabetic people in a Brazilian southeastern city. *Cien Saude Colet*. 2018;23(3):763–772. doi:10.1590/1413-81232018233.00752016
11. Hajian-Tilaki A, Oliae F, Jenabian N, Hajian-Tilaki K, Motallebnejad M. Oral health-related quality of life and periodontal and dental health status in Iranian hemodialysis patients. *J Contemp Dent Pract*. 2014;15(4):482–490. doi:10.5005/jp-journals-10024-1566
12. Machado V, Botelho J, Proença L, et al. Periodontal status, perceived stress, diabetes mellitus and oral hygiene care on quality of life: A structural equation modelling analysis. *BMC Oral Health*. 2020;20(1):229. doi:10.1186/s12903-020-01219-y
13. Mirzaii-Dizgah I, Agha-Hosseini F. Unstimulated whole saliva parathyroid hormone in postmenopausal women with xerostomia. *J Contemp Dent Pract*. 2011;12(3):196–199. doi:10.5005/jp-journals-10024-1034
14. Motallebnejad M, Hadian H, Mehdizadeh S, Hajiahmadi M. Validity and reliability of the Persian version of the oral health impact profile (OHIP)-14. *Caspian J Intern Med*. 2011;2(4):314–320. PMID:24551438. PMCID:PMC3895829.
15. Sadeghi R, Taleghani F, Farhadi S. Oral health related quality of life in diabetic patients. *J Dent Res Dent Clin Dent Prospects*. 2014;8(4):230–234. doi:10.5681/joddd.2014.41
16. Irani FC, Wassall RR, Preshaw PM. Impact of periodontal status on oral health-related quality of life in patients with and without type 2 diabetes. *J Dent*. 2015;43(5):506–511. doi:10.1016/j.jdent.2015.03.001
17. Mohamed HG, Mustafa K, Ibrahim SO, Åstrøm AN. Dietary habits, oral impact on daily performance and type 2 diabetes: A matched case–control study from Sudan. *Health Qual Life Outcomes*. 2017;15(1):111. doi:10.1186/s12955-017-0686-9
18. Azogui-Lévy S, Dray-Spira R, Attal S, Hartemann A, Anagnostou F, Azerad J. Factors associated with oral health-related quality of life in patients with diabetes. *Aust Dent J*. 2018;63(2):163–169. doi:10.1111/adj.12577
19. Cortelli SC, Costa FO, Gargioni-Filho A, et al. Impact of gingivitis treatment for diabetic patients on quality of life related to periodontal objective parameters: A randomized controlled clinical trial. *Arch Oral Biol*. 2018;86:80–86. doi:10.1016/j.archoralbio.2017.11.010
20. Thomson WM, Ibrahim H, Lyons KM, Foster Page LA, Hanlin SM. Personality, xerostomia and OHRQoL among 35–54-year-olds. *Acta Odontol Scand*. 2019;77(2):114–118. doi:10.1080/00016357.2018.1510138
21. Ahmad MS, Bhayat A, Zafar MS, Al-Samadani KH. The impact of hyposalivation on quality of life (QoL) and oral health in the aging population of Al Madinah Al Munawarrah. *Int J Environ Res Public Health*. 2017;14(4):445. doi:10.3390/ijerph14040445
22. Botelho J, Machado V, Proença L, et al. Perceived xerostomia, stress and periodontal status impact on elderly oral health-related quality of life: Findings from a cross-sectional survey. *BMC Oral Health*. 2020;20(1):199. doi:10.1186/s12903-020-01183-7
23. Nascimento ML, Farias AB, Carvalho AT, et al. Impact of xerostomia on the quality of life of patients submitted to head and neck radiotherapy. *Med Oral Patol Oral Cir Bucal*. 2019; 24(6):e770–e775. doi:10.4317/medoral.23131

Original paper

Prevalence of two-rooted and one-rooted mandibular canines with two root canals in Poland, assessed using CBCT: A preliminary study

Magdalena Piskórz[A–D], Karolina Futyma-Gąbka[C,D], Ingrid Różyło-Kalinowska[E,F]

Department of Dental and Maxillofacial Radiodiagnostics, Medical University of Lublin, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):233–238

Address for correspondence
Karolina Futyma-Gąbka
E-mail: lek.dent.karolina.futyma@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on January 3, 2022
Reviewed March 26, 2022
Accepted on March 28, 2022

Published online on June 21, 2023

Abstract

Background. The normal anatomy of mandibular canines presents with 1 root and 1 root canal. Two roots are found in approx. 2% of cases, and a bilateral configuration is even rarer. Canines with 2 root canals are found in around 15% of cases. Cone-beam computed tomography (CBCT) enables the detailed visualization of the teeth.

Objectives. The present study aimed to evaluate the prevalence of two-rooted mandibular canines and one-rooted mandibular canines with 2 root canals in a Polish population by using CBCT.

Material and methods. A total of 300 consecutive CBCT scans, taken for different clinical indications, were examined to assess permanent mandibular canine anatomy. The study group included 182 females and 118 males aged 12–86 years (mean age: 31.7 years).

Results. Among 600 cases, 27 two-rooted teeth were found (4.5%), and there were only 6 cases of one-rooted mandibular canines with 2 root canals (1.0%). Six cases of two-rooted canines had this configuration bilaterally, all in females. Five cases of canines with 2 root canals were found on the left side (83.3%). The predominance of the occurrence of two-rooted canines in females (81.5%) was strongly emphasized.

Conclusions. The prevalence of two-rooted mandibular canines in a Polish population, evaluated by means of CBCT, was higher, while the presence of 2 root canals was lower than in recent literature reports. There was no side predilection of two-rooted mandibular canines, although their occurrence was higher in females.

Keywords: CBCT, two-rooted canines, mandibular canines, one-rooted canines with two root canals

Cite as
Piskórz M, Futyma-Gąbka K, Różyło-Kalinowska I. Prevalence of two-rooted and one-rooted mandibular canines with two root canals in Poland, assessed using CBCT: A preliminary study. *Dent Med Probl.* 2023;60(2):233–238. doi:10.17219/dmp/147758

DOI
10.17219/dmp/147758

Introduction

Cone-beam computed tomography (CBCT) has become a meaningful tool for diagnosis and treatment planning in dentistry. CBCT images give the opportunity to analyze a case in 3 dimensions – sagittal, coronal and axial. In comparison with medical computed tomography (CT), CBCT is characterized by lower radiation doses, a shorter scan time, better accessibility, and easier interpretation in daily dental practice. Therefore, many dentists use this radiological method for advanced diagnosis and treatment risk assessment.

The proper evaluation of the number of roots or root canals contributes to successful endodontic treatment.[1] The Vertucci system is useful for classifying the configuration of root canals and divides them into 8 types: Type I – a single main canal is present starting from the pulp chamber to the root apex; Type II – 2 separate canals leave the pulp chamber, but join to form 1 canal toward the apex; Type III – 1 canal leaves the pulp chamber and divides into 2 smaller canals, which merge again later to exit as 1 canal; Type IV – 2 separate and completely distinct canals run from the pulp chamber to the apex; Type V – a single canal exits the pulp chamber and divides into 2 canals with separate apical foramina; Type VI – 2 separate canals join at the middle of the root to form 1 canal, which extends to just short of the apex and divides into 2 again; Type VII – the canal starts as single until the middle third of the root, then divides into 2 separate canals that rejoin after some distance, and then divide into 2 again near the apex; and Type VIII – the pulp chamber near the coronal portion divides into 3 separate canals extending to the apex.[1] Ordinola-Zapata et al. created a modified classification system based on micro-CT scans, and identified 37 types of root canal configuration.[2] Further studies are needed to evaluate the use of this classification in CBCT examinations.

Mandibular canines are mostly one-rooted, and according to Vertucci, the most common type is Type I.[1] However, although it seems easy to treat such a tooth, it can cause problems, and an unnoticed additional root canal may lead to treatment failure. In orthodontics, impacted two-rooted canines may also cause some difficulties during therapy.[3] Nowadays, there is a noticeable tendency to develop three-dimensional (3D) examinations to carry out the entire diagnosis and treatment planning without applying additional radiological methods. Moreover, CBCT also allows the analysis of the soft-tissue profile.[4,5] Canines are vital teeth, and their role is to provide structural and functional balance, tearing during mastication, and esthetic harmony. Thus, canines should be carefully analyzed to deliver the best treatment plan and keep them in the oral cavity as long as possible.

To the best of our knowledge, no study has evaluated the prevalence of an additional root or root canal in mandibular canines in Poland based on a CBCT examination. Therefore, the present study aimed to assess the prevalence of inferior two-rooted canines and one-rooted canines with 2 canals in a Polish population by using CBCT.

Material and methods

A total of 300 consecutive CBCT scans retrieved from the database of the Department of Dental and Maxillofacial Radiodiagnostics of the Medical University of Lublin, Poland, were analyzed to assess the anatomy of mandibular canines. The study group included only mature teeth, those at stage H according to Demirjian's tooth formation classification, and only the scans with the bilateral presence of canines. Endodontically treated teeth were excluded from the study, as were those with any signs of inflammation, resorption or apical cysts. Other exclusion criteria were the visible movement of the patient and the capping artifacts. The included canines had to be positioned correctly in the dental arch, without significant inclination. The examinations used the VistaVox S CBCT (Dürr Dental, Bietigheim-Bissingen, Germany), with a field of view (FOV) size of 130 mm × 85 mm and a slice thickness of 120 µm. Since some of the patients also

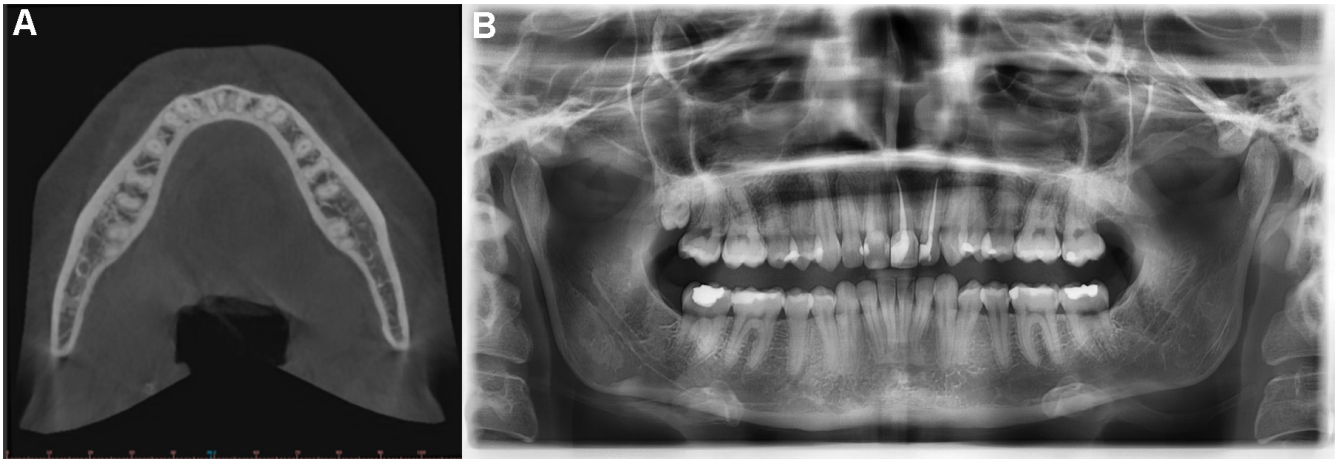

Fig. 1. Bilateral presence of two-rooted canines – cone-beam computed tomography (CBCT) axial view (A). The panoramic image does not provide the full visibility of two-rooted canines (B)

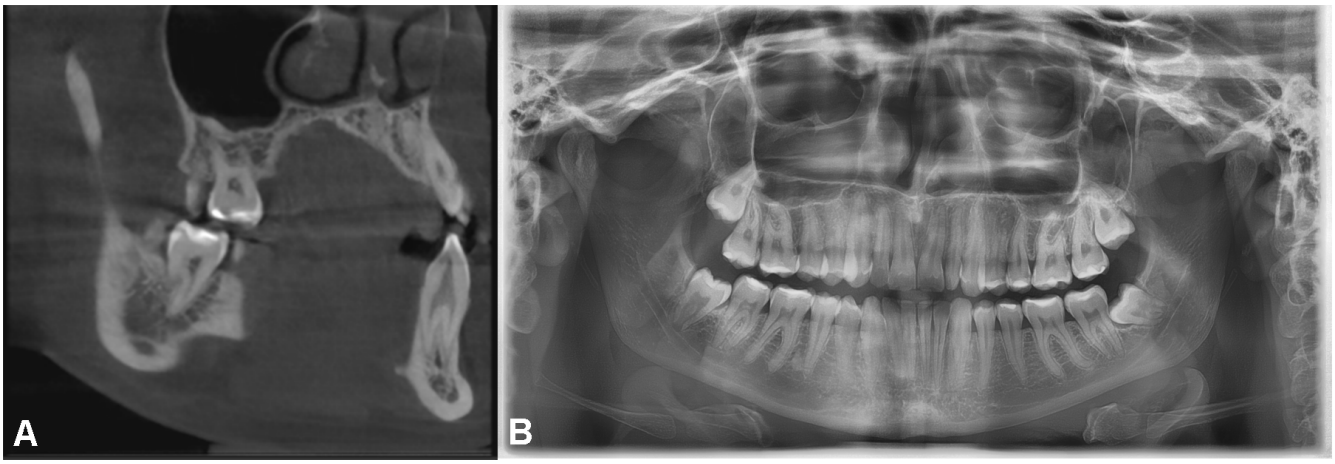

Fig. 2. Left mandibular two-rooted canine – cone-beam computed tomography (CBCT) cross-sectional view (A). The panoramic image does not provide the full visibility of a two-rooted canine (B)

had a panoramic X-ray performed, in the next step of our research, we will compare the visibility of two-rooted canines in both types of examination. Only the scans with a large FOV were included, as not all CBCT images with a FOV of 50 mm × 50 mm provided a clear bilateral view of canines. In such cases, we were unable to evaluate predilection in relation to the side.

The research group included 182 females and 118 males aged 12–86 years (mean age: 31.7 years). The CBCT scans were analyzed by 2 dentists (with 3 and 10 years of experience in the field of dental and maxillofacial radiodiagnostics) in multiplanar reconstructions (sagittal, coronal and axial) and oblique planes, using dedicated image processing software VistaSoft (Dürr Dental) and the Coronis Fusion 4MP radiological diagnostic display system (MDCC-4430; Barco, Kortrijk, Belgium). The examples of the scans are shown in Fig. 1A, 2A and 3.

Two examples of panoramic radiographs are presented in Fig. 1B and 2B to emphasize the validity of the study. The panoramic X-rays showed no clear radiological signs of two-rooted mandibular canines or one-rooted canines with two root canals, which were confirmed when using CBCT.

Results

Among 600 cases, we found 27 two-rooted teeth (4.5%) (Table 1). Six cases of two-rooted canines had a bilateral configuration, all in females (22.2%). The predominance of the occurrence of two-rooted canines in females (81.5%) was strongly emphasized (22/5). They were found on the right side more often.

Among all the evaluated teeth, only 6 cases of one-rooted mandibular canines with 2 root canals were found (1.0%), with 83.3% of them situated on the left side and 16.7% on the right side. Teeth with two root canals were found more often in females (66.7%) (Table 2). All cases (100%) were Type III according to Vertucci's classification.[1]

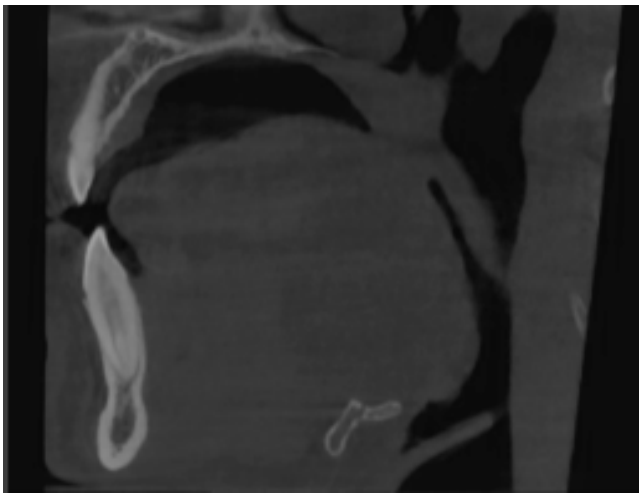

Fig. 3. Left mandibular canine with two root canals – cone-beam computed tomography (CBCT) cross-sectional view

Table 1. Prevalence of two-rooted mandibular canines

Two-rooted mandibular canines	Prevalence	
	F	M
Left (33)	4	2
Right (43)	6	3
Bilaterally (33 and 43)	6	0
Total	22	5

F – females; M – males.

Table 2. Prevalence of one-rooted mandibular canines with 2 root canals

One-rooted mandibular canines with 2 root canals	Prevalence	
	F	M
Left (33)	4	1
Right (43)	0	1
Bilaterally (33 and 43)	0	0
Total	4	2

When age was taken into consideration, the results showed that two-rooted canines and one-rooted canines with 2 root canals were most common in the patients aged 21–30 years. The age-related outcomes are presented in Tables 3 and 4.

Table 3. Presence of two-rooted mandibular canines in different age groups

Age group [years]	Number of two-rooted canines
12–20	0
21–30	18
31–40	4
41–50	2
51–60	3
61–70	0
71–80	0
81–86	0

Table 4. Presence of one-rooted mandibular canines with 2 root canals in different age groups

Age group [years]	Number of one-rooted canines with 2 root canals
12–20	0
21–30	5
31–40	1
41–50	0
51–60	0
61–70	0
71–80	0
81–86	0

Discussion

The detection of an additional root or root canal in mandibular canines influences the success of root canal treatment (RCT), but is also crucial in periapical surgery, periodontal treatment and the management of impacted teeth in orthodontics. Dentists must be aware of the anatomical alterations to the tooth to avoid iatrogenic failure. Researchers have applied different techniques and methods to evaluate external and internal tooth morphology, including staining and clearing,[6,7] periapical radiographs,[8] sectioning,[9] and micro-CT.[10,11] Recently, CBCT has been widely used in many studies investigating root and canal configuration because of its advantages.[12–16]

It is generally very rare for mandibular canines to have 2 roots and more than 1 root canal. Indeed, Type I is the most common type of lower canine, based on Vertucci's system.[1,17,18] Aminsobhani et al. found that 4.7% of mandibular canines were two-rooted,[13] which is similar to the results of our study (4.5%). Rahimi et al. presented a higher occurrence of 2 roots in lower canines in the examined population as compared to our study (12.08%).[19] Others, including Mashyakhy et al.,[16] Zhengyan et al.,[15] Kayaoglu et al.,[14] Karataşlioğlu et al.,[20] and Han et al.[21] found two-rooted canines in 2.7%, 0.8%, 3.1%, 3.4%, and 1.32% of patients, respectively. Pécora et al. performed a study based on extracted mandibular canines and found that only 1.7% of the examined sample were bifid teeth.[22] This variety of results can be caused by ethnic differences or smaller sample sizes. Interestingly, anthropological research revealed that the bi-rooted mandibular canine was a common feature in the European population between the 11th and 19th centuries, while no case was confirmed in the Asiatic population.[23]

The results of the present study showed a higher prevalence of two-rooted lower canines in females than in males (81.5%). This finding is in agreement with the results of studies performed by Karataşlioğlu et al.,[20] Doumani et al.,[12] Kayaoglu et al.,[14] and Mashyakhy et al.,[16] but it is not in agreement with a study by Soleymani et al., in which males presented with a higher prevalence of two-rooted canines than females.[24] A systematic review published in 2017 showed that the accessory root in lower canines is most common in females,[25] which is in agreement with our study.

The present study also aimed to evaluate bilateral symmetry in lower canine morphology. Our results showed a high level of symmetry for the number of roots (98.3%), which is similar to the outcomes of other research studies. Mashyakhy et al.[16] and Kayaoglu et al.[14] found that 95.5% of lower canines had a bilateral symmetry for the number of roots, which is also consistent with the result of 97.7% in a study performed by Al-Dahman et al.[26] Our study additionally assessed bilateral symmetry in two-rooted mandibular canines. Six cases revealed the presence of this configuration (22.2%), and all bilateral findings were found in females.

The assessment of the number of roots has been performed in various populations globally, but studies from Europe are scarce. It was concluded that the occurrence of bi-rooted mandibular canines was higher in a Turkish population[14,20] and lower in a Chinese population.[15,21] Studies performed on an Iranian population differ significantly in their results. Aminsobhani et al. found bifid roots only in 4.7% of their sample,[13] while Soleymani et al. found two-rooted canines only in 1.33%.[24]

The evaluation of tooth anatomy is also valuable in orthodontics. However, there is still a lack of research on bi-rooted canines in orthodontic management. In 2020, Raina and Goje presented a case report of impacted mandibular canines with 2 roots.[3] The authors emphasized that the number of roots influenced the anchor value of the tooth.[3] This can be significant in extraction space closure cases, such as bimaxillary protrusion treatment, in which 4 premolars are extracted and the anterior teeth are retracted into the obtained space. When a canine has 2 roots, it increases the overall root

surface area of the anterior segment and can cause some treatment difficulties.

The number of one-rooted lower canines with 2 canals in our study was low and equaled only 1.0%. This value is lower as compared to the data obtained by Zhengyan et al.[15] In that study, the prevalence of a second root canal in a mandibular canine was 2.4%.[15] In a study by Almohaimede et al., double canals were found in 9.94% of the examined canines.[27]

In the present study, most teeth had a Type I configuration, according to Vertucci.[1] This finding is similar to studies performed by Karataşlioğlu et al.,[20] Zhengyan et al.[15] and Soleymani et al.[24] In the current study, all 6 cases (100%) of one-rooted mandibular canines with 2 canals were Type III.

Limitations

The main limitation of this study was the small sample examined. Further research is required with an increased number of CBCT scans and the comparison of the visibility of canine anatomy with panoramic radiographs. Increased awareness of dentists about the possibility of the anatomical variability of the teeth can reduce the occurrence of treatment failure.

Conclusions

In our study, based on a CBCT examination, the majority of lower canines in a Polish population were single-rooted with a single canal. There was a higher prevalence of two-rooted canines in females than in males. Also, one-rooted canines with a double root canal were more common in females. CBCT proved to be a valuable tool for evaluating the number of roots and root canals in mandibular canines.

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

Magdalena Piskórz https://orcid.org/0000-0003-4092-1122
Karolina Futyma-Gąbka https://orcid.org/0000-0003-3415-8669
Ingrid Różyło-Kalinowska https://orcid.org/0000-0001-5162-1382

References

1. Vertucci FJ. Root canal morphology and its relationship to endodontic procedures. *Endod Topics*. 2005;10(1):3–29. doi:10.1111/j.1601-1546.2005.00129.x
2. Ordinola-Zapata R, Versiani MA, Bramante CM. Root canal components. In: Versiani M, Basrani B, Sousa-Neto M, eds. *The Root Canal Anatomy in Permanent Dentition*. Cham, Switzerland: Springer; 2018:31–46. doi:10.1007/978-3-319-73444-6_3
3. Raina P, Goje SK. A novel case report of impacted mandibular canine with bifid roots. *J Integr Health Sci*. 2020;8(2):86–90. doi:10.4103/JIHS.JIHS_18_20
4. Perrotti G, Baccaglione G, Clauser T, et al. Total Face Approach (TFA) 3D cephalometry and superimposition in orthognathic surgery: Evaluation of the vertical dimensions in a consecutive series. *Methods Protoc*. 2021;18;4(2):36. doi:10.3390/mps4020036
5. Alhammadi MS, Al-Mashraqi AA, Alnami RH, et al. Accuracy and reproducibility of facial measurements of digital photographs and wrapped cone beam computed tomography (CBCT) photographs. *Diagnostics (Basel)*. 2021;11(5):757. doi:10.3390/diagnostics11050757
6. Weng XL, Yu SB, Zhao SL, et al. Root canal morphology of permanent maxillary teeth in the Han nationality in Chinese Guanzhong area: A new modified root canal staining technique. *J Endod*. 2009;35(5):651–656. doi:10.1016/j.joen.2009.02.010
7. Dinakar C, Shetty UA, Salian VV, Shetty P. Root canal morphology of maxillary first premolars using the clearing technique in a South Indian population: An in vitro study. *Int J Appl Basic Med Res*. 2018;8(3):143–147. doi:10.4103/ijabmr.IJABMR_46_18
8. Pineda F, Kuttler Y. Mesiodistal and buccolingual roentgenographic investigation of 7,275 root canals. *Oral Surg Oral Med Oral Pathol*. 1972;33(1):101–110. doi:10.1016/0030-4220(72)90214-9
9. Weine FS, Healey HJ, Gerstein H, Evanson L. Canal configuration in the mesiobuccal root of the maxillary first molar and its endodontic significance. *Oral Surg Oral Med Oral Pathol*. 1969;28(3):419–425. doi:10.1016/0030-4220(69)90237-0
10. Versiani MA, Pécora JD, Sousa-Neto MD. The anatomy of two-rooted mandibular canines determined using micro-computed tomography. *Int Endod J*. 2011;44(7):682–687. doi:10.1111/j.1365-2591.2011.01879.x
11. Alkaabi W, AlShwaimi E, Farooq I, Goodis HE, Chogle SM. A micro-computed tomography study of the root canal morphology of mandibular first premolars in an Emirati population. *Med Princ Pract*. 2017;26(2):118–124. doi:10.1159/000453039
12. Doumani M, Habib A, Alhalak AB, Al-Nahlawi TF, Al Hussain F, Alanazi SM. Root canal morphology of mandibular canines in the Syrian population: A CBCT assessment. *J Family Med Prim Care*. 2020;9(2):552–555. doi:10.4103/jfmpc.jfmpc_655_19
13. Aminsobhani M, Sadegh M, Meraji N, Razmi H, Kharazifard MJ. Evaluation of the root and canal morphology of mandibular permanent anterior teeth in an Iranian population by cone-beam computed tomography. *J Dent (Tehran)*. 2013;10(4):358–366. PMID:24396355. PMCID:PMC3875510.
14. Kayaoglu G, Peker I, Gumusok M, Sarikir C, Kayadugun A, Ucok O. Root and canal symmetry in the mandibular anterior teeth of patients attending a dental clinic: CBCT study. *Braz Oral Res*. 2015;29:S1806-83242015000100283. doi:10.1590/1807-3107BOR-2015.vol29.0090
15. Zhengyan Y, Keke L, Fei W, Yueheng L, Zhi Z. Cone-beam computed tomography study of the root and canal morphology of mandibular permanent anterior teeth in a Chongqing population. *Ther Clin Risk Manag*. 2015;12:19–25. doi:10.2147/TCRM.S95657
16. Mashyakhy M. Prevalence of a second root and canal in mandibular and maxillary canines in a Saudi Arabian population: A cone-beam computed tomography study. *J Contemp Dent Pract*. 2019;20(7):773–777. PMID:31597794.
17. Vertucci FJ. Root canal anatomy of the human permanent teeth. *Oral Surg Oral Med Oral Pathol*. 1984;58(5):589–599. doi:10.1016/0030-4220(84)90085-9
18. Vertucci FJ. Root canal anatomy of the mandibular anterior teeth. *J Am Dent Assoc*. 1974;89(2):369–371. doi:10.14219/jada.archive.1974.0391
19. Rahimi S, Milani AS, Shahi S, Sergiz Y, Nezafati S, Lotfi M. Prevalence of two root canals in human mandibular anterior teeth in an Iranian population. *Indian J Dent Res*. 2013;24(2):234–236. doi:10.4103/0970-9290.116694

20. Karataşlioğlu E, Kalabalik F. Morphological evaluation of maxillary and mandibular canines using cone-beam computed tomography in Turkish population. *Ann Med Res*. 2019;26(10):2312–2319. doi:10.5455/annalsmedres.2019.09.525
21. Han T, Ma Y, Yang L, Chen X, Zhang X, Wang Y. A study of the root canal morphology of mandibular anterior teeth using cone-beam computed tomography in a Chinese subpopulation. *J Endod*. 2014;40(9):1309–1314. doi:10.1016/j.joen.2014.05.008
22. Pécora JD, Sousa Neto MD, Saquy PC. Internal anatomy, direction and number of roots and size of human mandibular canines. *Braz Dent J*. 1993;4(1):53–57. PMID:8180486.
23. Lee C, Scott GR. Brief communication: Two-rooted lower canines – a European trait and sensitive indicator of admixture across Eurasia. *Am J Phys Anthropol*. 2011;146(3):481–485. doi:10.1002/ajpa.21585
24. Soleymani A, Namaryan N, Moudi E, Gholinia A. Root canal morphology of mandibular canine in an Iranian population: A CBCT assessment. *Iran Endod J*. 2017;12(1):78–82. doi:10.22037/iej.2017.16
25. Plascencia H, Cruz Á, Gascón G, Ramírez B, Díaz M. Mandibular canines with two roots and two root canals: Case report and literature review. *Case Rep Dent*. 2017;2017:8459840. doi:10.1155/2017/8459840
26. Al-Dahman Y, Alqedairi A, Alfawaz H, Alnassar F, Al-Jebaly A. Cone-beam computed tomographic evaluation of root canal morphology of mandibular canines in a Saudi subpopulation. *Saudi Endod J*. 2019;9(2):113–118.
27. Almohaimede AA, Alqahtani AA, Alhatlani NM, Alsaloom NS, Alqahtani SA. Interpretation of root canal anatomy of maxillary and mandibular permanent canines in Saudi subpopulation: A cone-beam computed tomography (CBCT) study. *Int J Dent*. 2021;2021:5574512. doi:10.1155/2021/5574512

Original paper

Comparison of calcium sulfate and tricalcium phosphate in bone grafting after sinus lifting for dental implantation: A randomized controlled trial

Mohey Aldeen Amam[1,A,B,D], Anas Abdo[2,C], Amirah Alnour[3,E], Amam Amam[4,B], Mohamad Hassan Jaafo[1,F]

[1] Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Damascus University, Syria
[2] Department of Endodontics, Faculty of Dentistry, Damascus University, Syria
[3] Department of Oral Pathology, Faculty of Dentistry, Damascus University, Syria
[4] Department of Periodontics, Faculty of Dentistry, Damascus University, Syria

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):239–246

Address for correspondence
Amirah Alnour
E-mail: dr.amieranour@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on April 5, 2022
Reviewed on June 28, 2022
Accepted on July 8, 2022

Published online on June 23, 2023

Abstract

Background. Maxillary sinus grafting is considered the most common surgical technique to secure a sufficient bone height for placing dental implants. It is carried out either by making a bony window in the lateral wall of the maxillary sinus (the external procedure) or through the alveolar entrance technique by using alveolar osteotomes (the internal procedure), depending on the quality and quantity of the remaining bone.

Objectives. The aim of the present study was to compare radiologically the amount of bone gain (an increase in bone dimensions) and bone reduction (the loss of the graft volume) obtained by using tricalcium phosphate (TCP) and calcium sulfate (CS) grafts mixed with advanced platelet-rich fibrin (A-PRF).

Material and methods. Nine patients (18 maxillary sinuses) participated in this study, all of whom had bilateral edentulism involving the premolar/molar areas and a bone height of 0.5–5 mm between the sinus floor and the alveolar ridge. Two biomaterials were used in the sinus augmentation procedures. Each patient underwent a bilateral maxillary sinus lift with the use of different bone graft materials – with CS mixed with A-PRF used on one side, and TCP mixed with A-PRF on the other side. The grafting site was selected randomly. Afterward, bone gain and bone reduction were evaluated at the grafting site by using cone-beam computed tomography (CBCT).

Results. The mean bone gain on the side treated with TCP mixed with A-PRF was 7.532 ±1.150 mm, and on the side treated with CS mixed with A-PRF side it was 7.961 ±2.781 mm. The comparison of bone gain and bone reduction between the 2 groups showed no statistically significant differences at a 6-month follow-up.

Conclusions. Using CS or TCP mixed with A-PRF was beneficial and safe in the two-stage maxillary sinus lifting procedure. A sufficient amount of bone was obtained for dental implantation.

Keywords: dental implant, sinus lift, bone graft, calcium sulfate, tricalcium phosphate

Cite as
Amam MA, Abdo A, Alnour A, Amam A, Jaafo MH. Comparison of calcium sulfate and tricalcium phosphate in bone grafting after sinus lifting for dental implantation: A randomized controlled trial. *Dent Med Probl.* 2023;60(2):239–246. doi:10.17219/dmp/151983

DOI
10.17219/dmp/151983

Introduction

Bone height in the maxillary posterior edentulous area may be insufficient for dental implant placement due to the pneumatization of the maxillary sinus after the extraction of the teeth.[1] Maxillary sinus grafting is the most commonly used surgical technique for securing bone height sufficient for dental implantation in such cases.[2] The operation is carried out using one of the 2 basic techniques, i.e., making a bony window in the lateral wall of the maxillary sinus (the external sinus lift) or through the alveolar approach with the use of alveolar osteotomes (the internal sinus lift).[3]

Many types of bone grafts have been used, though autogenous bone has always been considered the gold standard due to superior osteoconduction, osteoinduction and osteogenesis.[4] However, there are some disadvantages connected with the process of acquiring it, including the limited amount of bone obtained from the inside of the oral cavity and the need for an additional surgical procedure, which leads to an increase in the surgery time. These factors have led to increasing interest in the search for alternative graft materials.[5] Tricalcium phosphate (TCP) is a bone substitute that promotes bone growth[4] and is considered one of the preferred grafts for maxillary sinus lifting due to its suitable absorption nature and volume stability.[6]

Calcium sulfate (CS) occupies a unique position in the field of regenerative materials, as it has a long history of clinical usage as compared to other currently available biomaterials and is widely recognized as a well-tolerated material with applications in bone regeneration. It undergoes virtually complete resorption in vivo, without eliciting a significant inflammatory response,[7–9] which is critical in this procedure, as the positioning of implants seems to be even more delicate. In particular, recent studies have highlighted extremely high levels of peri-implant tissue inflammation as compared to the natural tooth, which promotes long-term bone remodeling and resorption. Using drugs that contain inflammation-moderating components may also enhance the properies of the bone.[8]

Studies have investigated mixing alloplastic grafts with platelet-rich fibrin (PRF) to reduce the amount of graft and promote osteogenesis in the grafted area. The texture resulting from such mixing facilitates clinical handling, increases the stability of the graft and improves the outcomes.[10,11]

This study aimed to evaluate and compare the benefits of using CS and TCP as graft materials for the two-stage maxillary sinus lifting procedure in cases of high bone resorption, using the radiological analysis of bone gain and bone reduction.

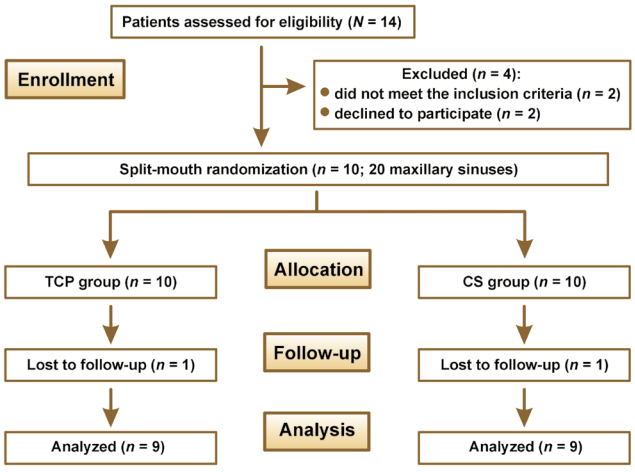

Fig. 1. CONSORT (Consolidated Standards of Reporting Trials) flow diagram
TCP – tricalcium phosphate; CS – calcium sulfate.

Material and methods

Study design

This was a randomized (1:1), split-mouth clinical trial (randomized controlled trial – RCT) (No. Faculty of Dentistry/RCTs-758) comparing the use of CS and TCP in bone grafting for external sinus lifting for dental implantation.

The Consolidated Standards of Reporting Trials (CONSORT) statement was used as a guide for this study.[12] The study was conducted in the laboratory of the Maxillofacial Surgery Hospital and the Department of Implantology at the Faculty of Dentistry of Damascus University, Syria (Fig. 1).

Informed consent was obtained from the participants, and the ethics board at the Faculty of Dentistry of Damascus University, Syria, approved the study (FMD-185).

Participants

The sample was selected from among the patients who sought implant treatment at the Department of Oral and Maxillofacial Surgery at the Faculty of Dentistry of Damascus University, Syria. Data was collected from February 2018 to January 2021.

Sample size calculation

The sample size was calculated using the G*Power 3.1.3 program (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower), based on a study power of 80% with a significance level at $p = 0.05$, and the effect size data (0.83) from a study by Călin et al.[13] Considering sample dropout led to the addition of 2 maxillary sinuses to each group to give a total sample size of 20 maxillary sinuses. The sample size for each group was 20 maxillary sinuses (10 patients).

Randomization

The maxillary sinuses were randomly allocated using Microsoft Excel 2010 to either the CS group (the intervention group) or the TCP group (the control group). Therefore, there were 10 maxillary sinuses per group.

The inclusion criteria comprised good oral health, bilateral edentulism in the maxilla, age between 45 and 70 years, and the bone height of the alveolar ridge between the alveolar crest and the bottom of the maxillary sinus ranging from 0.5 to 5 mm.

The exclusion criteria were as follows: metabolic diseases that affect normal bone metabolism, such as hyperparathyroidism or osteoporosis; being treated with drugs that cause bone metabolic disorders, such as corticosteroids, oral contraceptives, hormonal or chemical treatment, without ever having undergone radiotherapy to the head and neck region; general systemic diseases, such as diabetes, cardiovascular disorders, leukemia, hypertension, and coagulation disorders; autoimmune diseases; and any local contraindications, including the inflammation of the maxillary sinuses.

Methods

Primary stage

A cone-beam computed tomography (CBCT) image was taken before the commencement of the surgical procedure. This phase was considered as time zero (T0). The dental scaling of the jaws with the use of chlorhexidine (0.12%) rinses was performed 2 or 3 days prior to surgery. The medication (a 750 mg Levoflox (levofloxacin) tablet) was prescribed 24 h before surgery and 9 days post-surgery.

Second stage

Immediately before the surgical procedure, advanced platelet-rich fibrin (A-PRF) was prepared by aspirating 60–80 mL of blood from the patient's basilar vein in the elbow fold, using 20-milliliter syringes or 24-gauge intravenous catheters when a larger volume was required. The aspirated blood was placed in special A-PRF tubes and centrifuged immediately at 1,500 rpm for 14 min.[14]

Surgical method

The mouth was disinfected with 0.12% chlorhexidine rinses, the skin around the mouth was disinfected with a polyvidone iodine solution and the surgical area was isolated using sterile surgical scrubs. Local (buccal and palatal) anesthesia utilized 2% lidocaine hydrochloride (HCl) and epinephrine (1:80,000) (Fig. 2). A trapezoid-shaped, full-thickness mucoperiosteal buccal flap was then created (Fig. 3).

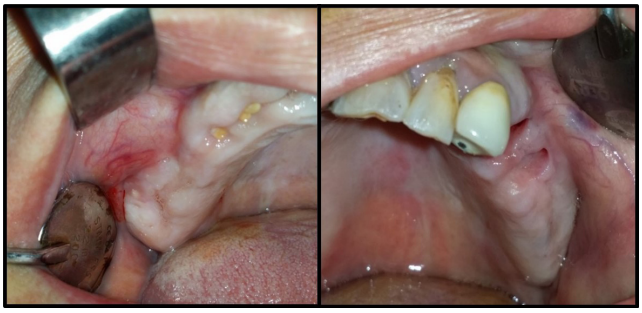

Fig. 2. Bilaterally edentulous maxilla before surgery and after anesthesia

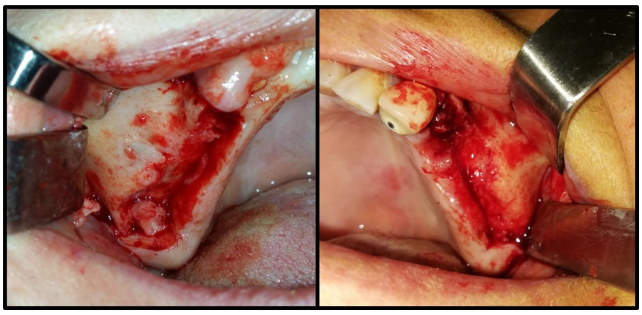

Fig. 3. Elevation of a full-thickness flap

Using a piezosurgical device with appropriate saline irrigation, a bony window with rounded corners was created to reduce perforation during lifting. It had dimensions of 12–15 mm in length and 10 mm in height based on the size of the area to be grafted. A CBCT radiograph indicates the thickness of the bony window, which facilitates its preparation; 2–3 mm above the bottom of the maxillary sinus to enable sufficient vision during work and reduce the tension of the sinus membrane in the initial lifting phase. However, the window should not be enlarged much, as the surrounding walls aid bone healing (Fig. 4).

The sinus membrane was elevated with a sinus lift tool – the Dentium Advanced Sinus Kit (DASK) (Dentium, Cypress, USA), and for the grafting of the maxillary sinus, the mixture of CS or TCP with A-PRF was used (Fig. 5). When using the TCP/A-PRF compound, we placed a collagen membrane on the bony window (Fig. 6). The CS/A-PRF compound does not require any membrane, as a catalyst is added to the CS graft to harden fast. As such, the CS graft replaces the membrane due to its hardening and slow absorption properties.[15]

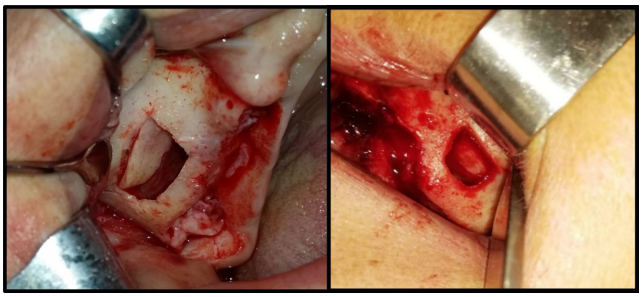

Fig. 4. Elevating the sinus membrane (the window raised and kept attached to the membrane)

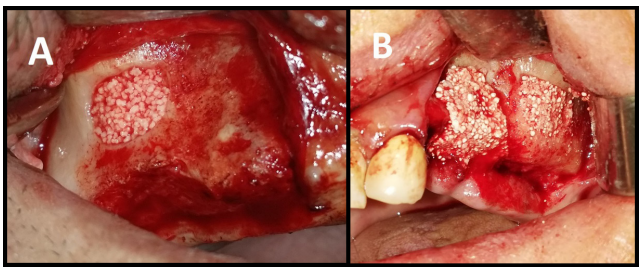

Fig. 5. Filling the space created by raising the maxillary sinus with the 2 grafts used in the study

A – TCP with advanced platelet-rich fibrin (A-PRF); B – CS with A-PRF.

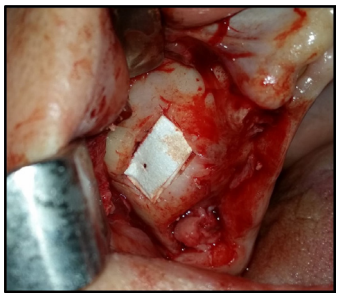

Fig. 6. Application of a collagen membrane on the bony window after the placement of the tricalcium phosphate (TCP)/advanced platelet-rich fibrin (A-PRF) graft

Interrupted suturing employed 4-0 Prolene sutures and a reverse cutting needle (Ethicon US, Cincinnati, USA) (Fig. 7). The patients received post-surgery instructions and a medical prescription, and had a follow-up appointment to remove the sutures (Fig. 8).

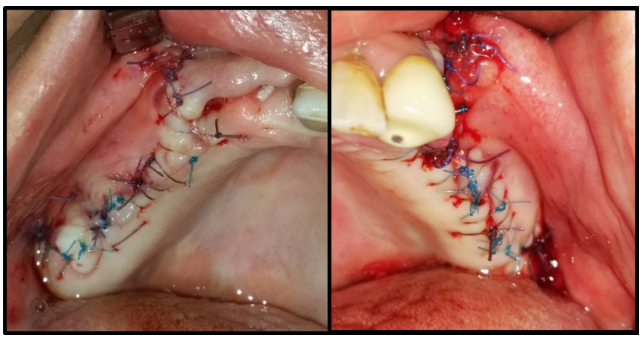

Fig. 7. Wound closure with Proline sutures

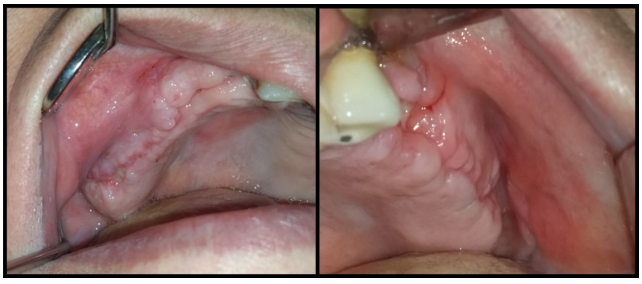

Fig. 8. Removal of the sutures at a follow-up

Radiological study method

Three CBCT radiographs were performed for each patient with the use of the PaX-i3D Green imaging system (Vatech, Hwaseong, South Korea). All scans were conducted in the same radiology center to standardize the characteristics of the radiographs, with the same position being repeated pre-op (T0), immediately post-op (T1) and 6 months post-op (T2), i.e., before the 2nd surgical operation (implantation).

The radiographs were examined using the OnDemand3D program (https://www.ondemand3d.com/en), which enabled standardization by merging the 2 radiographs (taken at T0 and T1) to ensure the measurements of the same site on both scans, and to avoid any changes that could be caused by altering the position of the patient's head (Fig. 9).

The 1st image (T0) allowed the measurement of bone height before the maxillary sinus lift, using points in the sagittal view. The 3rd image (T2) was used to measure the amount of lifting at the same sites.

Bone height was measured on the 1st image (T0) at 5 locations in the coronal view, where each point in the coronal view matched the corresponding point in the sagittal view, using the 'ruler' tool. Bone height was also measured on the 3rd image (T2) at 5 locations in the coronal view. Using the same method, we measured bone height in the sagittal and coronal views on the 2nd image (T1).

The following measurements were calculated: bone height immediately after surgery; bone gain after 6 months; and bone reduction after 6 months (Equations 1,2):

$$\text{bone gain} = \begin{matrix}\text{bone height}\\ \text{after 6 months}\end{matrix} - \begin{matrix}\text{bone height}\\ \text{before lifting}\end{matrix} \quad (1)$$

$$\begin{matrix}\text{bone}\\ \text{reduction}\end{matrix} = \begin{matrix}\text{height of the bone and the graft}\\ \text{immediately after lifting}\end{matrix} - \begin{matrix}\text{bone height}\\ \text{after 6 months}\end{matrix} \quad (2)$$

Statistical analysis

The IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA) was used to perform all statistical analyses, and a p-value of 0.05 was considered statistically significant. The Shapiro–Wilk test determined the normality of data distribution, and the independent t test evaluated differences between the 2 groups at T0, T1 and T2.

The null hypotheses were as follows:

- There is no statistical difference between T0 and T2 in bone height in the TCP group (1).
- There is no statistical difference between T0 and T2 in bone height in the CS group (2).
- There is no statistical difference between the TCP and CS groups when comparing bone gain (3).
- There is no statistical difference between the TCP and CS groups when comparing bone reduction (4).

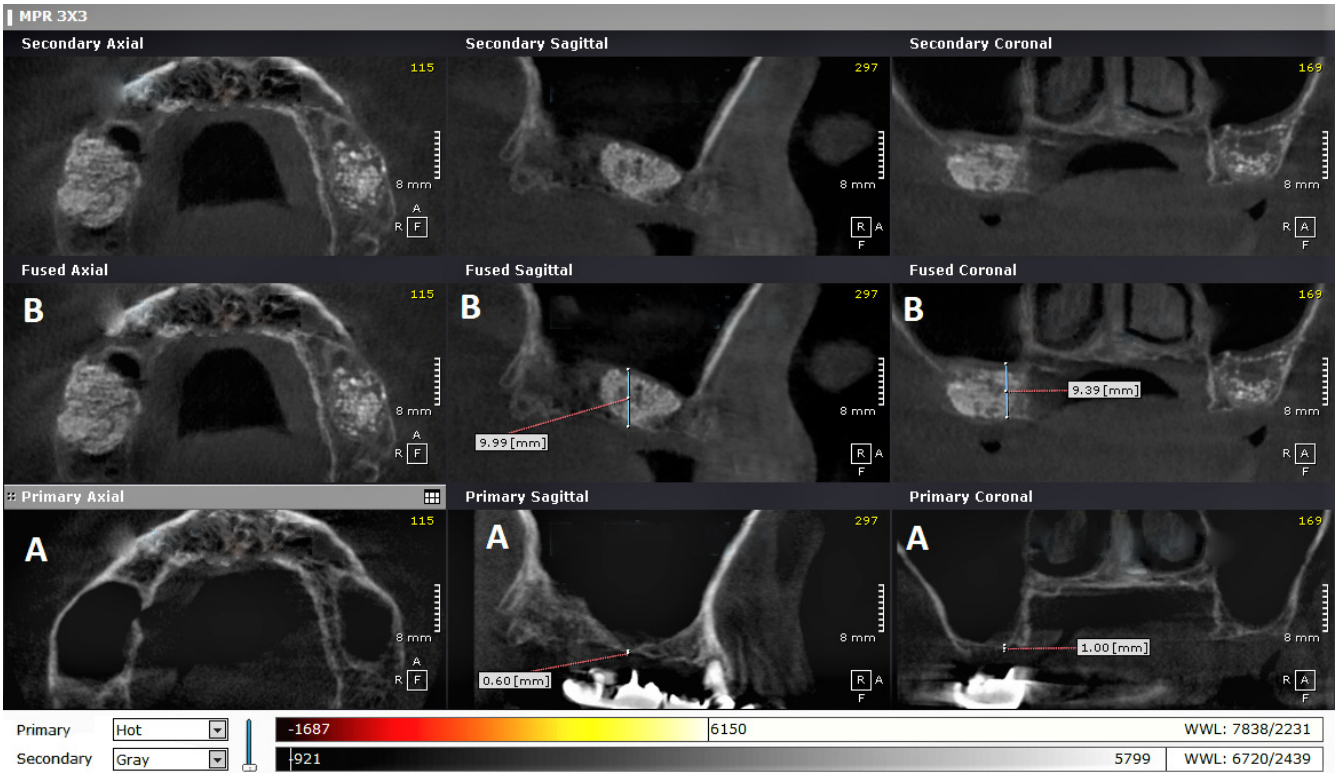

Fig. 9. Merged images obtained by using the OnDemand3D program
A – before surgery (T0); B – 6 months after the sinus lift (T2).

Error of the method

A total of 25% of the measurements were randomly selected and repeated a month after the 1st measurement by the same examiner (MAA). Systematic and random errors were calculated by comparing the 1st and 2nd measurements with the use of the paired *t* test. No statistically significant differences were found between the 1st and 2nd measurements for any variable ($p > 0.05$).

Results

Figure 1 shows the CONSORT flow diagram. The study included 20 maxillary sinuses in 10 patients, though 1 patient was excluded from the sample after refusing to attend the radiographic follow-up. Therefore, a complete follow-up was done for 9 patients (18 maxillary sinuses), and the statistical analysis was conducted. The descriptive statistics of bone height in both groups at each time stage are shown in Table 1.

The results presented in Tables 2 and 3 indicate that bone height increased significantly in the TCP and CS groups between T0 and T1 and between T0 and T2, and decreased significantly between T1 and T2 ($p < 0.05$).

Table 4 shows that there were no significant differences, at a confidence interval (*CI*) of 95%, in the amount of bone gain ($p = 0.693$) or bone reduction ($p = 0.678$) between the 2 groups. The mean bone gain in the CS graft

Table 1. Mean bone height values [mm] in both groups at each time stage

Graft	Time stage	*M* ±*SD*	min	max
TCP	T0	3.859 ±1.728	0.968	5.932
	T1	14.185 ±3.025	10.987	20.577
	T2	11.391 ±0.934	10.162	12.527
CS	T0	3.545 ±2.131	1.114	7.181
	T1	13.858 ±1.966	11.675	17.742
	T2	11.506 ±2.440	7.891	15.290

Time stages: T0 – pre-op; T1 – immediately post-op; and T2 – 6 months post-op.
M – mean; *SD* – standard deviation; min – minimum, max – maximum.

Table 2. Changes in bone height at different time stages in the tricalcium phosphate (TCP) group (paired *t* test)

Time stage	*MD*	*t*-value	*p*-value
T0–T1	10.326	14.281	0.000*
T0–T2	7.532	18.547	0.000*
T1–T2	2.794	3.410	0.011*

MD – mean difference; * statistically significant ($p < 0.05$).

Table 3. Changes in bone height at different time stages in the calcium sulfate (CS) group (paired *t* test)

Time stage	*MD*	*t*-value	*p*-value
T0–T1	10.313	10.965	0.000*
T0–T2	7.961	8.096	0.000*
T1–T2	2.352	3.629	0.008*

* statistically significant ($p < 0.05$).

Table 4. Comparison of the amount of bone gain and bone reduction in the 2 study groups (independent *t* test)

Bone change	*MD*	*t*-value	*p*-value
Bone gain	0.429	0.403	0.693
Bone reduction	0.442	0.424	0.678

was higher than in the TCP graft, with a minor difference recorded (0.43 mm). Also, the mean bone reduction in the TCP graft was slightly greater than in the CS graft, with a 0.44 mm difference between the two.

Discussion

Maxillary sinus lifting is a still evolving procedure, necessary to increase bone height for dental implants. However, performing the procedure requires extended knowledge of maxillary sinus anatomy and its variations, as there may occur difficulties in window preparation, causing the perforation of the Schneiderian membrane. Before sinus lift surgery, CBCT should be conducted to discover the prevalence of septa.[16]

Many studies have investigated the optimal bone graft for sinus lifting, although autogenous bone grafts are considered the gold standard, as they provide osteoconduction, osteoinduction and osteogenesis – the 3 essential elements for bone regeneration.[16] However, the additional surgical site created to obtain an autograft increases the procedure time and causes more pain to the patient.[16,17] These factors have led to increasing interest in the search for alternative graft materials, mixing autografts with other types of bone grafts or completely replacing them with other grafts.[9]

There is increased interest in using alloplastic bone grafts to facilitate surgical procedures. However, the large amounts of materials required in cases of high maxillary sinus bone absorption increase expense. Therefore, studies have investigated mixing alloplasts with A-PRF to reduce the amount of graft needed and promote bone graft osteogenesis. Advanced PRF is an autologous graft material that eliminates any risk of disease transmission. In addition, its gelatinous consistency improves clot and graft stability, as it reduces the time required to ossify.[11]

Based on the current literature, the present research aimed to investigate the effectiveness of the CS/A-PRF compound in the grafting procedure after a maxillary sinus lift. Calcium sulfate is readily available, provides acceptable results, is easy to use, and helps to reduce surgical costs. Also, unlike other bone substitutes,[18,19] it can be used without absorbable and non-absorbable membranes.[18]

To verify the CS graft results, it was compared with TCP, as it is the same class of alloplast, it is reliable and is frequently used in the grafting procedure,[6] and provides a resorbable scaffold for bone growth.[9] Both grafts were mixed with A-PRF to accelerate and increase new bone formation, and reduce the amount of graft material used.[11] Since there are no previous studies in the literature comparing these 2 grafts, this study aimed to compare CS/A-PRF with TCP/A-PRF in the external sinus lift procedures, using the radiographic measurements of bone gain and bone reduction after 6 months.

The study sample included 20 maxillary sinuses of 10 patients who had a bilaterally edentulous posterior maxilla, were aged 45–70 years, had bone height between the alveolar bone crest and the bottom of the maxillary sinus ranging from 0.5 mm to 5 mm (class SA4 according to Misch classification), did not suffer from any systemic diseases affecting the surgical procedure, and did not suffer from any health problems in the nose and sinuses that are considered a contraindication for sinus lifting. One patient was excluded from the sample, because he refused to attend the radiographic follow-up. Therefore, the sample consisted of 18 maxillary sinuses (9 patients) randomly distributed into 2 groups, with 9 sinuses in the CS group and 9 sinuses in the TCP group. A split-mouth technique was used, where the CS/A-PRF graft was applied to one maxillary sinus, and the TCP/A-PRF graft was applied to the other side.

The lateral approach technique was followed to lift the maxillary sinus, since this method is indicated for maxillary sinus elevating in class SA4 cases and the delayed implantation excludes other methods, such as the alveolar approach (the internal sinus lift).[20] In addition, the implant success rate is higher in the two-stage method than in the one-stage technique in SA4 cases.[21]

An ultrasonic system was used for window preparation, similar to other studies,[22] as it reduces the incidence of the perforation of the maxillary sinus membrane to 7% from the 25% experienced with rotary instruments. The method also reduces pain, discomfort and edema after surgery, and generally helps to protect soft tissues, including the maxillary sinus membrane and the mandibular nerve.[23]

After maxillary sinus grafting, the bony window was covered with an absorbable collagen membrane on the side grafted with the TCP/A-PRF compound, which helps to prevent the surrounding connective tissue cells from entering the bone graft material and increases vital bone formation.[24] No membrane was used for the CS/A-PRF compound, and the window was covered only with the CS graft material. The material can be used as a membrane due to its physical properties,[15] which reduces the financial cost and the surgical procedure time.

The radiological study used CBCT images, which are accurate and sufficient for determining reference points, and three-dimensional (3D) measurements can be made with ease. Three radiographs were performed for each patient to study bone gain and bone reduction in the grafted area,[25] with the 1st one taken pre-op (T0), the 2nd immediately post-op (T1) and the 3rd 6 months post-op (T2).

The CS/A-PRF graft material proved to be useful and safe for the two-stage external maxillary sinus lifting

procedure, as bone sufficient for dental implant placement was obtained after 6 months. The mean bone height at T0 was 3.545 ±2.131 mm, which increased immediately to 13.858 ±1.966 mm at T1. At T2, bone height decreased to 11.506 ±2.440 mm, giving a gain of 7.961 ±2.781 mm and a reduction of 2.352 ±1.832 mm.

Guarnieri et al.[8] and Tarnow et al.[26] reported that CS grafts promoted implant stability and new bone formation after its absorption. However, a study that followed up 2 years after the 1st surgery found a greater reduction than the current study (1.0–3.5 mm), perhaps due to the difference in the observation period (2.5 years).[15]

The use of TCP/A-PRF as a grafting material after external sinus lifting helped to secure a sufficient amount of bone for implantation, where the average bone height at T0 was 3.859 ±1.728 mm and it increased immediately to the size of the graft at T1 (14.185 ±3.025 mm). After 6 months, bone height decreased to 11.391 ±0.934 mm, resulting in a gain of 7.532 ±1.150 mm and a reduction of 2.794 ±2.310 mm. Oba et al. recorded a gain of 3.11 ±1.35 mm, which is less than in this study, perhaps because their study used the osteotome sinus lifting technique,[27] which does not allow to achieve a high bone gain as compared to the lateral lifting method.[25] Okada et al. also found a reduction of 0.73 ±1.33 mm, which was due to the differences in the radiological and surgical methods, where the implants were placed at the same time as grafting.[28]

When comparing the 2 study groups, we did not find significant differences in the amount of bone gain or bone reduction, and no clinical or radiological complications were observed during the 6-month follow-up period.

Conclusions

Both grafts can be used for maxillary sinus lifting with the delayed implantation. The properties of CS are negatively affected by moisture, so the graft must be applied with a pasty texture in addition to isolating the receiving area, and it is better to apply the material in layers to reduce shrinkage. The A-PRF material helped to increase the graft size, reduced costs and promoted bone formation, while it also helped to increase TCP graft bonding.

Ethics approval and consent to participate

Informed consent was obtained from the participants, and the ethics board at the Faculty of Dentistry of Damascus University, Syria, approved the study (FMD-185).

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

Mohey Aldeen Amam https://orcid.org/0000-0001-8533-6520
Anas Abdo https://orcid.org/0000-0001-9702-2887
Amirah Alnour https://orcid.org/0000-0002-4626-4082
Amam Amam https://orcid.org/0000-0002-5063-4947
Mohamad Hassan Jaafo https://orcid.org/0000-0002-0602-9678

References

1. Sharan A, Madjar D. Maxillary sinus pneumatization following extractions: A radiographic study. *Int J Oral Maxillofac Implants*. 2008;23(1):48–56. PMID:18416412.
2. Sorní M, Guarinós J, García O, Peñarrocha M. Implant rehabilitation of the atrophic upper jaw: A review of the literature since 1999. *Med Oral Patol Oral Cir Bucal*. 2005;10(Suppl 1):E45–E56. PMID:15800467.
3. Fugazzotto PA, Vlassis J. Long-term success of sinus augmentation using various surgical approaches and grafting materials. *Int J Oral Maxillofac Implants*. 1998;13(1):52–58. PMID:9509780.
4. Misch CE, Dietsh F. Bone-grafting materials in implant dentistry. *Implant Dent*. 1993;2(3):158–167. doi:10.1097/00008505-199309000-00003
5. Farina R, Pramstraller M, Franceschetti G, Pramstraller C, Trombelli L. Alveolar ridge dimensions in maxillary posterior sextants: A retrospective comparative study of dentate and edentulous sites using computerized tomography data. *Clin Oral Implants Res*. 2011;22(10):1138–1144. doi:10.1111/j.1600-0501.2010.02087
6. Olaechea A, Mendoza-Azpur G, Valle FO, Padial-Molina M, Martin-Morales N, Galindo-Moreno P. Biphasic hydroxyapatite and β-tricalcium phosphate biomaterial behavior in a case series of maxillary sinus augmentation in humans. *Clin Oral Implants Res*. 2019;30(4):336–343. doi:10.1111/clr.13419
7. Iezzi G, Fiera E, Scarano A, Pecora G, Piattelli A. Histologic evaluation of a provisional implant retrieved from man 7 months after placement in a sinus augmented with calcium sulphate: A case report. *J Oral Implantol*. 2007;33(2):89–95. doi:10.1563/0.808.1
8. Guarnieri R, Zanza A, D'Angelo M, et al. Correlation between peri-implant marginal bone loss progression and peri-implant sulcular fluid levels of metalloproteinase-8. *J Pers Med*. 2022;12(1):58. doi:10.3390/jpm12010058
9. Bouwman WF, Bravenboer N, Frenken JW, Ten Bruggenkate CM, Schulten EA. The use of a biphasic calcium phosphate in a maxillary sinus floor elevation procedure: A clinical, radiological, histological, and histomorphometric evaluation with 9- and 12-month healing times. *Int J Implant Dent*. 2017;3(1):34. doi:10.1186/s40729-017-0099-x
10. Chen H, Cui X, Yu XZ, et al. Effects of chitosan-coated pressed calcium sulfate pellets combined with recombinant human bone morphogenetic protein 2 on bone formation in femoral condyle-contained bone defects. *J Craniofac Surg*. 2010;21(1):188–197. doi:10.1097/SCS.0b013e3181c50f8f
11. Ghanaati S, Booms P, Orlowska A, et al. Advanced platelet-rich fibrin: A new concept for cell-based tissue engineering by means of inflammatory cells. *J Oral Implantol*. 2014;40(6):679–689. doi:10.1563/aaid-joi-D-14-00138
12. Schulz KF, Altman DG, Moher D; CONSORT group. CONSORT 2010 statement: Updated guidelines for reporting parallel group randomised trials. *BMC Med*. 2010;8:18. doi:10.1186/1741-7015-8-18
13. Călin DL, Rusu A, Mitrea M. Sinus lift using a mixture of A-PRF and cerabone and simultaneous insertion of a single implant. *Romanian Journal of Functional and Clinical, Macro- and Microscopical Anatomy and of Anthropology/Revista Română de Anatomie Funcţională şi Clinică, Macro şi Microscopică şi Antropologie* (*Rev Rom Anat Funcţ Clin Macro Microsc Antropol*). 2016;15(1):115–122.
14. Nóbrega C, Koga da Silva EM, de Macedo CR. Low-level laser therapy for treatment of pain associated with orthodontic elastomeric separator placement: A placebo-controlled randomized double-blind clinical trial. *Photomed Laser Surg*. 2013;31(1):10–16. doi:10.1089/pho.2012.3338

15. Bagio DA, Julianto I, Suprastiwi E, Margono A. Ideal concentration of advanced-platelet rich fibrin (A-PRF) conditioned media for human dental pulp stem cells differentiation. *Pesqui Bras Odontopediatria Clin Integr*. 2019;19(1):e4754. doi:10.4034/PBOCI.2019.191.109
16. Malec M, Smektala T, Tutak M, Trybek G, Sporniak-Tutak K. Maxillary sinus septa prevalence and morphology-computed tomography based analysis. *Int J Morphol*. 2015;33(1):144–148. http://www.intjmorphol.com/wp-content/uploads/2015/07/art_23_331.pdf. Accessed January 1, 2022.
17. Bathla SC, Fry RR, Majumdar K. Maxillary sinus augmentation. *J Indian Soc Periodontol*. 2018;22(6):468–473. doi:10.4103/jisp.jisp_236_18
18. Moy PK, Lundgren S, Holmes RE. Maxillary sinus augmentation: Histomorphometric analysis of graft materials for maxillary sinus floor augmentation. *J Oral Maxillofac Surg*. 1993;51(8):857–862. doi:10.1016/s0278-2391(10)80103-x
19. Beretta M, Cicciù M, Bramanti E, Maiorana C. Schneider membrane elevation in presence of sinus septa: Anatomic features and surgical management. *Int J Dent*. 2012;2012:261905. doi:10.1155/2012/261905
20. Yahav A, Kurtzman GM, Katzap M, Dudek D, Baranes D. Bone regeneration: Properties and clinical applications of biphasic calcium sulfate. *Dent Clin North Am*. 2020;64(2):453–472. doi:10.1016/j.cden.2019.12.006
21. Orsini M, Orsini G, Benlloch D, et al. Comparison of calcium sulfate and autogenous bone graft to bioabsorbable membranes plus autogenous bone graft in the treatment of intrabony periodontal defects: A split-mouth study. *J Periodontol*. 2001;72(3):296–302. doi:10.1902/jop.2001.72.3.296
22. Misch CE, Perel ML, Wang HL, et al. Implant success, survival, and failure: The International Congress of Oral Implantologists (ICOI) Pisa Consensus Conference. *Implant Dent*. 2008;17(1):5–15. doi:10.1097/ID.0b013e3181676059
23. Wannfors K, Johansson B, Hallman M, Strandkvist T. A prospective randomized study of 1- and 2-stage sinus inlay bone grafts: 1-year follow-up. *Int J Oral Maxillofac Implants*. 2000;15(5):625–632. PMID:11055129.
24. Doan NV, Huynh TQ, Tran S, et al. Multidisciplinary approach to maximize angiogenesis and wound healing using piezoelectric surgery, concentrated growth factors and photobiomodulation for dental implant placement surgery involving lateral wall sinus lift: Two case reports. *VascularCell*. 2020;12(1):2. doi:10.24238/13221-12-1-186
25. Al-Dajani M. Recent trends in sinus lift surgery and their clinical implications. *Clin Implant Dent Relat Res*. 2016;18(1):204–212. doi:10.1111/cid.12275
26. Tarnow DP, Wallace SS, Froum SJ, Rohrer MD, Cho SC. Histologic and clinical comparison of bilateral sinus floor elevations with and without barrier membrane placement in 12 patients: Part 3 of an ongoing prospective study. *Int J Periodontics Restorative Dent*. 2000;20(2):117–125. PMID:11203554.
27. Oba Y, Tachikawa N, Munakata M, Okada T, Kasugai S. Evaluation of maxillary sinus floor augmentation with the crestal approach and beta-tricalcium phosphate: A cone-beam computed tomography 3- to 9-year follow-up. *Int J Implant Dent*. 2020;6(1):27. doi:10.1186/s40729-020-00225-7
28. Okada T, Kanai T, Tachikawa N, Munakata M, Kasugai S. Long-term radiographic assessment of maxillary sinus floor augmentation using beta-tricalcium phosphate: Analysis by cone-beam computed tomography. *Int J Implant Dent*. 2016;2(1):8. doi:10.1186/s40729-016-0042-6

Original paper

Comparative study of changes in the airway dimensions following the treatment of Class II malocclusion patients with the twin-block and Seifi appliances

Soodeh Tahmasbi[1,A,F], Massoud Seifi[1,B], Ali Asghar Soleymani[2,E], Fatemeh Mohamadian[2,C], Mostafa Alam[3,D,E]

[1] Department of Orthodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Pediatric Dentistry, School of Dentistry, Qom University of Medical Sciences, Iran
[3] Department of Oral and Maxillofacial Surgery, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):247–254

Address for correspondence
Mostafa Alam
E-mail: mostafa_alam1@yahoo.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on April 23, 2021
Reviewed on July 28, 2021
Accepted on September 15, 2021

Published online on June 29, 2023

Abstract

Background. Functional appliances are frequently used to stimulate mandibular growth in cases of Class II malocclusion with mandibular deficiency. Many studies have reported improved pharyngeal airway passage (PAP) dimensions following functional appliance therapy in children.

Objectives. The present study aimed to assess changes in the airway dimensions following the treatment of Class II malocclusion patients with the twin-block and Seifi appliances.

Material and methods. Lateral cephalograms of 37 patients with Class II malocclusion and mandibular deficiency treated with the twin-block appliance ($n = 20$) or the Seifi appliance ($n = 17$) were assessed in this before-and-after study. The preoperative and postoperative lateral cephalograms were compared to determine changes in the airway dimensions at the level of the palatal plane (PP), the occlusal plane (OP) and the 2nd–4th cervical vertebrae (C2–C4) in the 2 groups. The results were analyzed with the t test and the one-way analysis of covariance (ANCOVA).

Results. After treatment, significant changes occurred in the point A–nasion–point B (ANB) and sella–nasion–point B (SNB) skeletal cephalometric indices in the twin-block appliance group, and in ANB, SNB and incisor–mandibular plane angle (IMPA) in the Seifi appliance group. The airway dimensions at the level of PP, OP and the 3rd cervical vertebra (C3) significantly increased postoperatively as compared to the baseline in the twin-block appliance group ($p < 0.05$). The increases in the airway dimensions at the level of PP and C3 in the twin-block appliance group were significantly greater than in the Seifi appliance group ($p < 0.05$).

Conclusions. The treatment of Class II Division I malocclusion with the twin-block appliance significantly increased the airway dimensions at the level of PP, OP and C3, whereas the Seifi appliance did not cause any significant changes in the airway dimensions.

Keywords: malocclusion, airways, orthodontic appliances, Angle's Class II, functional

Cite as
Tahmasbi S, Seifi M, Soleymani AA, Mohamadian F, Alam M. Comparative study of changes in the airway dimensions following the treatment of Class II malocclusion patients with the twin-block and Seifi appliances. *Dent Med Probl.* 2023;60(2):247–254. doi:10.17219/dmp/142292

DOI
10.17219/dmp/142292

Introduction

The prevalence of dental and skeletal malocclusion is variable in different populations.[1] A meta-analysis conducted on the Iranian population reported a 24.7% prevalence of Class II malocclusion, ranking it second in terms of prevalence after Class I malocclusion.[2] Class II malocclusion is caused by mandibular deficiency in 65% of cases, and is due to maxillary prognathism in only a small percentage.[1,3] Mandibular deficiency can also occur due to the small size of the mandible or its retruded position relative to the maxilla.[4]

The growth and development of dentofacial structures and the pharyngeal airway dimensions have a mutual cause-and-effect relationship, i.e., the inhibited or limited growth of the craniofacial structures can lead to pharyngeal airway narrowing, and also a reduction in the nasopharyngeal airway dimensions due to anatomical obstruction can alter the craniofacial growth.[5,6] In Class II skeletal malocclusion caused by a retrognathic mandible, decreased space between the cervical vertebrae and the mandible body leads to airway narrowing, as well as a retrognathic position of the tongue and soft tissues,[7] which increases the risk of impairment in the respiratory function during the day and sleep-disordered breathing at night.[8] Decreased airway dimensions in childhood due to fat deposition in the posterior pharyngeal area increases the risk of sleep-disordered breathing in adulthood.[8,9]

The early orthodontic treatment of skeletal and dental anomalies during the primary dentition and early mixed dentition period aims to prevent the development or aggravation of anomalies in the late mixed dentition and permanent dentition periods, and decrease or eliminate the need for future treatment.[10] Functional appliances are used to modify the process of development of Class II malocclusion, mainly during the growth period, by changing the pattern of the remaining facial growth or altering the position of the jaw. Functional appliances improve the sensory proprioceptive feedback mechanisms of various perioral muscles that control the position and function of the mandible, and transfer the loads to the basal bone and the teeth.[4]

The treatment of Class II malocclusion not only corrects the skeletal facial structure, but also affects the posterior airway dimensions and can aid in the treatment of obstructive sleep apnea. Although some previous studies reported significant effects of the twin-block appliance on the pharyngeal airway dimensions,[1,11] others refuted such effects.[12] Considering the existing controversy in the available literature on changes in the airway dimensions following the use of the twin-block appliance, and a lack of studies on the effects of the Seifi functional appliance on the airway dimensions, the present study aimed to assess changes in the airway dimensions following the treatment of Class II Division I malocclusion patients with the twin-block and Seifi appliances.

Material and methods

This experimental before-and-after study was conducted on 37 patients with Class II malocclusion and a retrognathic mandible reporting to the Department of Orthodontics at the School of Dentistry of Shahid Beheshti University of Medical Sciences and a private dental office in Tehran, Iran. The study protocol was approved by the ethics committee at Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1397.036). Informed written consent was obtained from all the participants.

The inclusion criteria were as follows: age between 8 and 14 years; having Class II Division I malocclusion with a retrognathic mandible (sella–nasion–point B angle (SNB) ≤76°); a normal position of the maxilla (sella–nasion–point A angle (SNA) of 79–84°); a bilateral Class II molar relationship; incisor–mandibular plane angle (IMPA) of more than 85° and less than 90°; an overjet of more than 4 mm and less than 10 mm; mild or no crowding; no excess space in the arch; profile improvement when the patient was asked to protrude the mandible with an edge-to-edge position of the teeth; and treatment with the twin-block appliance or the Seifi appliance (Fig. 1). Patients with a history of orthodontic treatment or upper airway surgical procedures, those with craniofacial syndromes, and patients with a history of systemic diseases affecting the skeletal growth or the response to orthodontic treatment were excluded.

The minimum sample size was calculated to be 17, according to a previous study,[13] assuming $\alpha = 0.05$, $\beta = 0.20$ and a study power of 80%. The patients were selected by convenience sampling.

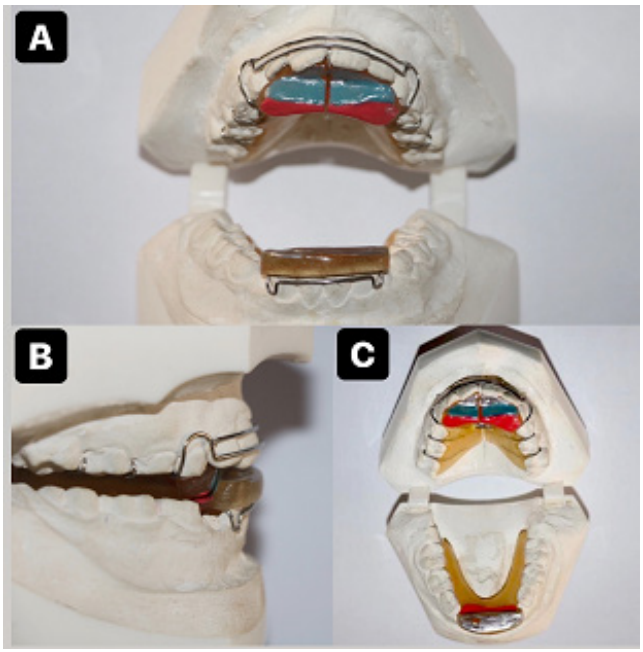

Fig. 1. Seifi appliance
A – frontal view; B – lateral view; C – occlusal view.

After obtaining the records of 37 eligible patients from the archives, they were assigned to the twin-block appliance treatment group (n = 20) or the Seifi appliance treatment group (n = 17). There were 9 females and 11 males aged 8–14 years in the twin-block appliance group, and 9 females and 8 males aged 9–12 years in the Seifi appliance group. Preoperative and postoperative patient records, including panoramic radiographs, lateral cephalograms, and intraoral and extraoral photographs, were retrieved from the archives.

This study adopted the airway analysis used by Kinzinger et al.,[14] with all the preoperative and postoperative lateral cephalograms of the patients in both groups analyzed by a third-year postgraduate student of pediatric dentistry after calibration with an orthodontist. The analysis of dental and skeletal features employed the Dolphin software, v. 10.1 (Dolphin Imaging & Management Solutions, Chatsworth, USA). Figure 2 shows the cephalometric landmarks used for this purpose. The airway dimensions were evaluated by hand tracing, and the related landmarks, reference planes and linear parameters are shown and described in Fig. 3 and 4 and Table 1. A cephalogram ruler was used to calibrate tracing in the Dolphin software and for manual tracing. Ten lateral cephalograms were randomly selected and traced twice by a third-year postgraduate student of pediatric dentistry, with a 2-week interval between the assessments. The intra-examiner reliability was assessed by calculating the intraclass correlation coefficient.

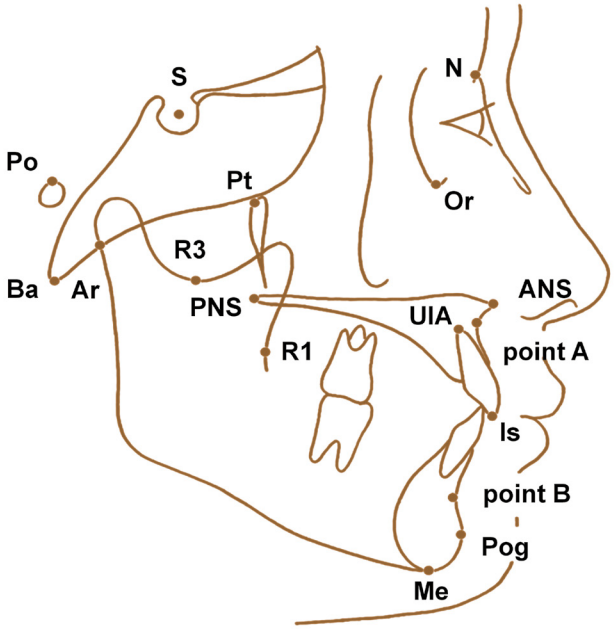

Fig. 2. Cephalometric landmarks

Ar – articulare; Ba – basion; Is – incision superius; Me – mention; N – nasion; S – sella; Or – orbitale; Po – porion; Pog – pogonion; Pt – pterygoid; R1 – ramus point 1; R3 – ramus point 3; UIA – upper incisor root apex.

Table 1. Cephalometric landmarks, reference planes and linear parameters used for the evaluation of changes in the airway dimensions

Indices		Definition
Landmark	P1	intersection of the palatal plane (PP) and the posterior pharyngeal wall
	P2	intersection of the occlusal plane (OP) and the posterior pharyngeal wall
	AP1	intersection of the occlusal plane and the uvula (the posterior border of the soft palate)
	AP2	intersection of the 2nd cervical vertebral plane and the dorsal surface of the tongue
	PP2	intersection of the 2nd cervical vertebral plane and the posterior pharyngeal wall
	AP3	intersection of the 3rd cervical vertebral plane and the base of the tongue
	PP3	intersection of the 3rd cervical vertebral plane and the posterior pharyngeal wall
	AP4	intersection of the 4th cervical vertebral plane and the anterior pharyngeal wall
	PP4	intersection of 4th cervical vertebral plane and the posterior pharyngeal wall
Reference plane	2nd cervical vertebral plane	line connecting AP2 and PP2
	3rd cervical vertebral plane	line connecting AP3 and PP3
	4th cervical vertebral plane	line connecting AP4 and PP4
Linear parameter	palatal plane (PP)	line connecting the anterior nasal spine (ANS) and the posterior nasal spine (PNS)
	occlusal plane (OP)	line connecting the center point of the orbit and the most distal contact point of posterior teeth
	AWPP	distance between PNS and P1
	AWOP	distance between AP1 and P2
	AWC2	distance between AP2 and PP2
	AWC3	distance between AP3 and PP3
	AWC4	distance between AP4 and PP4

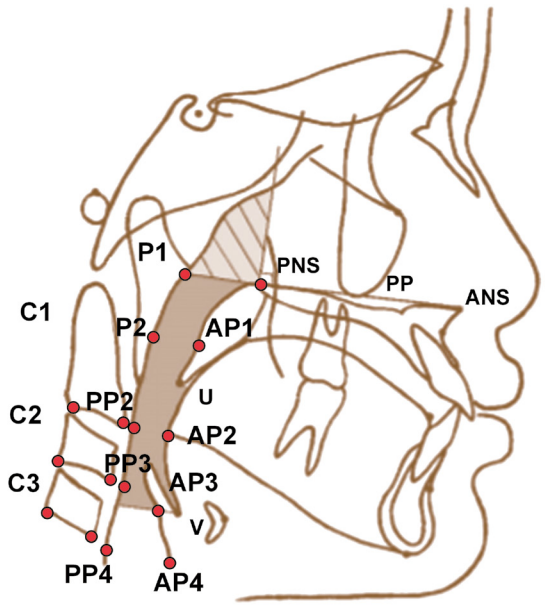

Fig. 3. Cephalometric landmarks used for the evaluation of changes in the airway dimensions

C1 – 1st cervical vertebra; C2 – 2nd cervical vertebra; C3 – 3rd cervical vertebra; U – tip of the soft palate; V –vallecular.

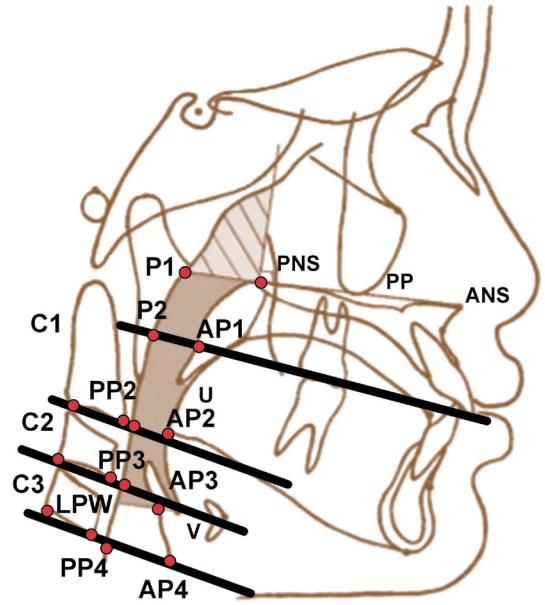

Fig. 4. Airway width at different levels according to the study by Kinzinger et al.[14]

LPW – lower pharyngeal wall.

Statistical analysis

All cephalometric indices were measured on preoperative and postoperative lateral cephalograms, and compared within each group with the use of the paired *t* test, while the independent *t* test and the analysis of covariance (ANCOVA) were used to compare differences between the groups. All statistical analyses employed IBM SPSS Statistics for Windows, v. 21.0 (IBM Corp., Armonk, USA), with a significance level at $p < 0.05$.

Results

Out of the 37 Class II Division I patients evaluated in this study, 20 were treated with the twin-block appliance and 17 with the Seifi appliance. The χ^2 test revealed no significant difference in gender distribution between the 2 groups ($p > 0.05$) and the independent *t* test showed no significant difference in the mean age between the 2 groups ($p > 0.05$). The duration of treatment was 6–36 months with the twin-block appliance and 12–36 months with the Seifi appliance, though the difference in this respect between the 2 groups was not significant ($p > 0.05$).

The intraclass correlation coefficient was 0.9 for the analysis of skeletal and dental cephalometric landmarks, and the airway dimensions, which was favorable.

Table 2 shows the preoperative and postoperative cephalometric indices for the 2 groups. The within-group comparisons with the use of the paired *t* test showed a significant increase in SNB and a significant decrease in ANB after treatment with the twin block appliance as compared to the baseline ($p < 0.05$). No other significant change was noted in this group ($p > 0.05$). In the Seifi appliance group, the paired *t* test showed that SNB and IMPA significantly increased, while ANB significantly decreased after treatment as compared to the baseline ($p < 0.05$). Other indices did not show a significant change in this group ($p > 0.05$).

Table 3 shows the comparisons of the mean changes in the skeletal cephalometric indices between the 2 groups. As shown, the maxillary incisor to sella–nasion angle (U1–SN) significantly decreased in the Seifi appliance

Table 2. Preoperative and postoperative cephalometric indices for the 2 groups

Appliance	Index	Before treatment	After treatment	*p*-value
Twin-block appliance	ANB [°]	6.2 ±2.8	3.7 ±1.7	<0.001*
	SNB [°]	75.9 ±4.5	77.6 ±4.4	0.010*
	SNA [°]	81.9 ±5.1	81.3 ±6.0	0.540
	IMPA [°]	99.5 ±8.0	101.2 ±5.9	0.210
	Jarabak index	69.2 ±12.1	65.9 ±5.3	0.230
	MP–SN [°]	32.5 ±5.3	31.5 ±6.5	0.320
	U1–SN [°]	106.2 ±7.6	107.1 ±6.4	0.240
Seifi appliance	ANB [°]	6.9 ±2.7	5.2 ±1.9	<0.001*
	SNB [°]	75.4 ±3.3	76.7 ±2.4	0.020*
	SNA [°]	82.3 ±4.5	82.2 ±3.0	0.900
	IMPA [°]	95.6 ±2.7	98.6 ±4.6	0.001*
	Jarabak index	62.4 ±3.7	62.9 ±2.9	0.400
	MP–SN [°]	37.2 ±4.4	37.2 ±4.6	0.900
	U1–SN [°]	100.2 ±8.2	96.1 ±11.2	0.070

Data presented as mean ± standard deviation (*M* ±*SD*). ANB – point A–nasion–point B angle; SNB – sella–nasion–point B angle; SNA – sella–nasion–point A angle; IMPA – incisor–mandibular plane angle; MP–SN – mandibular plane to sella–nasion angle; U1–SN – upper incisor to sella–nasion angle; * statistically significant.

Table 3. Comparisons of the mean changes in the skeletal cephalometric indices between the 2 groups

Index	Twin-block appliance	Seifi appliance	*p*-value†	*p*-value‡
ANB [°]	–2.5 ±2.4	–1.7 ±1.5	0.270	0.015*
SNB [°]	1.7 ±2.6	1.3 ±2.2	0.730	0.560
SNA [°]	–0.6 ±4.4	–0.1 ±3.0	0.660	0.580
IMPA [°]	1.7 ±6.2	3.0 ±2.9	0.420	0.030*
Jarabak index	–3.3 ±11.7	0.5 ±3.0	0.210	0.210
MP–SN [°]	–1.0 ±4.5	0.0 ±3.2	0.430	0.260
U1–SN [°]	0.9 ±5.8	–4.1 ±8.5	0.040*	0.010*

Data presented as *M* ±*SD*. * statistically significant; † independent *t* test; ‡ ANCOVA for the comparison of the postoperative values between the 2 groups after controlling for the effects of the preoperative values.

group, while it slightly increased in the twin-block appliance group. According to the independent *t* test, the change in this index was significant only in the Seifi appliance group ($p < 0.05$). The changes in other indices were not significant ($p > 0.05$).

Since differences in some indices (the Jarabak index (the ratio of the posterior facial height, measured as the distance between S and gonion (Go), to the anterior facial height, measured as the distance between N and menton (Me)), U1–SN and the mandible plane to sella–nasion angle (MP–SN)) were significant between the 2 groups at the baseline, ANCOVA was applied to control for the effects of such confounding variables. ANCOVA showed that, after eliminating the effects of the confounding variables, there were significant differences between the 2 groups with regard to the changes in ANB, IMPA and U1–SN ($p < 0.05$). No other significant differences were noted ($p > 0.05$).

Table 4 shows the within-group comparisons of the airway cephalometric indices before and after treatment in the 2 groups. As indicated, the paired *t* test demonstrated no significant change in any index after treatment with the Seifi appliance ($p > 0.05$). However, all indices significantly changed after treatment with the twin-block appliance ($p < 0.05$), except for AWC2 and AWC4, which increased insignificantly ($p > 0.05$).

Table 5 compares the mean changes in the airway cephalometric indices between the 2 groups. The independent *t* test revealed greater increases in all indices in the twin-block appliance group as compared to the Seifi appliance group after treatment, except for AWC2. However, these differences were significant only for AWPP and AWC3 ($p < 0.05$).

Since differences in some indices were significant between the 2 groups also before treatment, ANCOVA was applied to control for the effects of such confounding variables. Accordingly, the results showed significant differences in AWPP and AWC3 between the 2 groups ($p < 0.05$). No other significant differences were noted ($p > 0.05$).

Table 4. Airway cephalometric indices before and after treatment in the 2 groups

Appliance	Index	Before treatment	After treatment	*p*-value
Twin-block appliance	AWPP [mm]	14.7 ±5.5	18.3 ±4.5	0.005*
	AWOP [mm]	10.4 ±3.3	12.7 ±2.4	0.010*
	AWC2 [mm]	11.1 ±2.9	12.0 ±4.1	0.400
	AWC3 [mm]	10.4 ±3.9	14.3 ±5.4	0.010*
	AWC4 [mm]	13.3 ±4.3	14.4 ±4.7	0.190
Seifi appliance	AWPP [mm]	16.0 ±6.7	15.9 ±4.4	0.940
	AWOP [mm]	12.1 ±4.6	12.5 ±4.6	0.690
	AWC2 [mm]	10.7 ±2.6	12.2 ±3.3	0.160
	AWC3 [mm]	12.1 ±4.1	11.6 ±3.4	0.610
	AWC4 [mm]	12.8 ±2.9	13.0 ±2.9	0.700

Data presented as *M* ±*SD*. * statistically significant.

Table 5. Comparisons of the mean changes in the airway cephalometric indices between the 2 groups

Index	Twin-block appliance	Seifi appliance	*p*-value†	*p*-value‡
AWPP [mm]	3.6 ±5.0	–0.1 ±6.0	0.050*	0.040*
AWOP [mm]	2.3 ±3.6	0.4 ±4.7	0.200	0.550
AWC2 [mm]	0.9 ±4.4	1.5 ±4.3	0.640	0.770
AWC3 [mm]	3.9 ±5.9	–0.5 ±4.2	0.010*	0.040*
AWC4 [mm]	1.1 ±3.8	0.2 ±3.2	0.230	0.150

Data presented as *M* ±*SD*. * statistically significant; † independent *t* test; ‡ ANCOVA for the comparison of the postoperative values between the 2 groups after controlling for the effects of the preoperative values.

Discussion

This study compared changes in the airway dimensions following the treatment of Class II Division I malocclusion patients with the twin-block and Seifi appliances. The results showed significant increases in the airway dimensions at the level of the palatal plane (PP), the occlusal plane (OP) and the 3rd cervical vertebra (C3) following the use of the twin-block appliance, while the Seifi appliance caused insignificant increases in the airway dimensions at the level of OP, the 2nd cervical vertebra (C2) and the 4th cervical vertebra (C4). Such greater changes in the airway dimensions following the use of the twin-block appliance as compared to the Seifi appliance were mainly due to skeletal changes and can be attributed to a better correction of the retrognathic position of the mandible in Class II patients, which is reflected in a greater change in ANB. The retrognathic position of the tongue in patients with a retrognathic mandible pushes soft tissues backward and decreases the airway dimensions.[15] The forward repositioning of the mandible by functional appliances corrects the position of the hyoid bone and the tongue, and affects airway morphology.[16]

Another reason for increased airway dimensions following the treatment with functional appliances is a reduced thickness of the posterior pharyngeal wall.[17] However, other studies showed the inefficacy of functional appliances, and some even demonstrated an increased posterior pharyngeal wall thickness following treatment with such appliances.[18] Zhang et al. reported that treatment with functional appliances preserved the thickness of the posterior pharyngeal wall in the nasopharynx, oropharynx and hypopharynx, while the thickness decreased in most control patients.[19] These observations revealed that a reduction in the posterior pharyngeal wall thickness in the upper airways is a compensatory mechanism in patients with a retrognathic mandible who received no treatment to maintain an open airway. The present study also showed insignificant reductions in the airway dimensions at the level of PP and C3 following the use of the Seifi appliance, which may be due to an increased thickness of the posterior pharyngeal wall.

The comparison of changes in the airway dimensions between the 2 groups revealed significant increases in the airway dimensions at the level of PP, OP and C3 in the twin-block appliance group. Although some studies reported increased airway dimensions following the treatment of Class II malocclusion with functional appliances, this effect was transient; in contrast, some other studies demonstrated that this increase was significant in the long term.[11,20]

The patients evaluated in the present study were in their growth period. Thus, natural patient growth and the use of orthodontic appliances influenced the linear and angular changes. Assessing the pure effect of treatment alone requires a control group. However, the ethical legitimacy of having a control group in such studies is questionable, since depriving Class II malocclusion patients of treatment would be unethical. Although Buschang et al.[21] and Bishara et al.[22] found insignificant differences in the growth patterns of normal-occlusion and Class II malocclusion patients, Björk and Skieller found some significant differences in this respect between normal-occlusion and Class II malocclusion patients.[23] To decrease the effect of inter-individual differences in growth patterns on the results, the 2 groups were standardized for age and gender in the present study.

Lateral cephalograms are commonly used to assess the airway dimensions, as the skull is in a fixed position, and the patient is conscious and can maintain a natural head position. Thus, lateral cephalograms can be used reliably for assessing changes in skeletal and soft tissue structures.[12,24] However, lateral cephalometry provides two-dimensional (2D) images of three-dimensional (3D) structures.[20] The images are sharp in the midsagittal plane; nonetheless, transverse plane distances cannot be accurately measured. Thus, lateral cephalometry cannot provide precise information on the airway width. Also, lateral cephalometry has limitations with regard to measuring the posterior airway space. To overcome such limitations, cone-beam computed tomography (CBCT) or magnetic resonance imaging (MRI) can be used.[14] However, such imaging modalities should only be requested by physicians when medically indicated. Considering the retrospective design of this study, the available lateral cephalograms of the patients were retrieved from the archives and evaluated, as they are still commonly used for linear airway measurements.[14] The technique described by Kinzinger et al.[14] was adopted for the measurements in this study. Also, as the posterior border of the tongue cannot be easily detected on lateral cephalograms. The index described by Rose et al.[25] was used for assessing the airways at the level of OP.

The present study showed that the horizontal intermaxillary relationship significantly improved in both groups due to significant changes in ANB and SNB. Changes in IMPA and U1–SN demonstrate the effects of upper and lower teeth on overjet reduction, but in the twin-block appliance group, such changes. However, IMPA significantly increased after treatment with the Seifi appliance, and although the U1–SN reduction was not significant in this group, the *p*-value was close to the significance level ($p = 0.070$). Considering the greater skeletal changes in the sagittal plane following treatment with the twin-block and Seifi appliances, it appears that the efficacy of these appliances for overjet improvement is mainly due to skeletal changes. The comparison of the effects of the 2 appliances by means of the independent *t* test and ANCOVA revealed a significant reduction in U1–SN in the Seifi appliance group as compared to the twin-block appliance group. Moreover, ANCOVA showed a greater increase in IMPA following treatment with the Seifi appliance and a greater reduction in ANB in the twin-block appliance group, which were both significant. According to the results, skeletal changes in the sagittal plane were greater when using the twin-block appliance. In the vertical dimension, changes in the Jarabak index and MP–SN were not significant in any group. The Jarabak index decreased in the twin-block appliance group, but not significantly.

The decrease in ANB and the increase in SNB following treatment with the twin-block appliance are in agreement with the results of Ahmadian-Babaki et al.[15] and Toth and McNamara.[16] Parkin et al. also reported an increase in SNB, but it was not statistically significant.[17] However, LaHaye et al. reported no SNB change following treatment with the Herbst appliance.[18] In the present study, the change in U1–SN was not significant in the twin-block appliance group, while Ajami et al. reported a significant reduction of this index.[26] An increase in IMPA after treatment was reported in studies by de Almeida-Pedrin et al.,[27] O'Brien et al.,[13] Seifi et al.,[28] and Jamilian et al.[29] Ahmadian-Babaki et al. reported an insignificant reduction in the Jarabak index in the twin-block appliance group.[15]

The current study also showed a significant increase in the upper and middle airway dimensions in the twin-

block appliance group, which is in line with the results of Vinoth et al.,[24] while Ali et al.[1] and Jena et al.[30] only reported an increase in the upper airway dimensions.

The twin-block appliance repositions the mandible forward, and thus affects the position of the hyoid bone and the tongue. Such a change can affect the soft palate and subsequently improve the upper airway dimensions. The improvement in the upper airway dimensions with the twin-block appliance in the present study is in agreement with the results of Jena et al.;[30] however, Fastuca et al. reported no change in the airway dimensions after mandibular repositioning and maxillary expansion following treatment with the twin-block appliance.[31]

Ghodke et al. reported a significant increase in SNB of 1.8°, and increases in the depth of the nasopharynx (1.54 mm) and hypopharynx (1.77 mm).[32] Thapa et al. reported a significant increase of 1.97 mm in the depth of the oropharynx.[33] In the present study, the twin-block appliance treatment caused a significant increase of 1.7° in SNB, and nasopharynx and oropharynx depth increases of 3.6 mm and 2.3 mm, respectively. Kinzinger et al. showed insignificant reductions in the airway dimensions at the level of PP and C3 after treatment with the functional mandibular advancer,[14] which is in agreement with the results obtained in the present study for the Seifi appliance group. Yassaei et al. demonstrated significant increases in the airway dimensions and the changed position of the tongue and the hyoid bone after treatment with the Farmand appliance.[34]

Although functional appliances cause various skeletal and dental changes, they have limited effects on the airway dimensions. Kinzinger et al. compared the Herbst appliance and the functional mandibular advancer,[14] Godt et al. compared the Harvold activator and the bite-jumping appliance,[35] and Gu et al. compared the Herbst appliance and the twin-block applaince in terms of airway changes,[20] and none of them found significant differences between the appliances. However, the present study revealed significantly greater increases in the airway dimensions at the level of PP and C3 when using the twin-block appliance as compared to the Seifi appliance. Jena et al. compared the twin-block appliance and the mandibular protraction appliance-IV, and reported an increase in the airway depth in the hypopharynx in the twin-block appliance group, with the mandibular protraction appliance causing greater dental rather than skeletal changes, and having no significant effect on the airway dimensions.[30]

It is noteworthy that statistically significant changes as a result of using functional devices may not be clinically significant in some cases.[13]

Future studies with larger sample sizes are required to compare the effects of the Seifi appliance with other treatment modalities on the airway dimensions in Class II malocclusion patients. Also, future studies may use more advanced 3D imaging modalities, such as CBCT, for this purpose.

Conclusions

The twin-block appliance caused greater increases in the airway dimensions than the Seifi appliance. These results may help orthodontists to select an appliance that is functionally and structurally suitable (the mandibular growth being the primary aim) based on the effects it has on the structures supporting the upper airways.

Ethics approval and consent to participate

The study protocol was approved by the ethics committee at Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1397.036). Informed written consent was obtained from all the participants.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Soodeh Tahmasbi https://orcid.org/0000-0001-9016-7733
Massoud Seifi https://orcid.org/0000-0002-5976-7340
Ali Asghar Soleymani https://orcid.org/0000-0003-4454-5582
Fatemeh Mohamadian https://orcid.org/0000-0002-8055-8762
Mostafa Alam https://orcid.org/0000-0003-3827-9708

References

1. Ali B, Shaikh A, Fida M. Changes in oro-pharyngeal airway dimensions after treatment with functional appliance in Class II skeletal pattern. *J Ayub Med Coll Abbottabad*. 2015;27(4):759–763. PMID:27004316.
2. Akbari M, Lankarani KB, Honarvar B, Tabrizi R, Mirhadi H, Moosazadeh M. Prevalence of malocclusion among Iranian children: A systematic review and meta-analysis. *Dent Res J (Isfahan)*. 2016;13(5):387–395. doi:10.4103/1735-3327.192269
3. Hamid WU. Prevalence of skeletal components of malocclusion using composite cephalometric analysis. *Pak Oral Dent J*. 2003;23(2):137–144. https://vlibrary.emro.who.int/imemr/prevalence-of-skeletal-components-of-malocclusion-using-composite-cephalometric-analysis. Accessed April 1, 2021.
4. Kannan A, Sathyanarayana HP, Padmanabhan S. Effect of functional appliances on the airway dimensions in patients with skeletal Class II malocclusion: A systematic review. *J Orthod Sci*. 2017;6(2):54–64. doi:10.4103/jos.JOS_154_16
5. Linder-Aronson S, Leighton BC. A longitudinal study of the development of the posterior nasopharyngeal wall between 3 and 16 years of age. *Eur J Orthod*. 1983;5(1):47–58. doi:10.1093/ejo/5.1.47
6. Ceylan I, Oktay H. A study on the pharyngeal size in different skeletal patterns. *Am J Orthod Dentofacial Orthop*. 1995;108(1):69–75. doi:10.1016/s0889-5406(95)70068-4
7. Harvold EP, Chierici G, Vargervik K. Experiments on the development of dental malocclusions. *Am J Orthod*. 1972;61(1):38–44. doi:10.1016/0002-9416(72)90174-1
8. Schafer ME. Upper airway obstruction and sleep disorders in children with craniofacial anomalies. *Clin Plast Surg*. 1982;9(4):555–567. doi:10.1016/S0094-1298(20)31948-9

9. Ozbek MM, Miyamoto K, Lowe AA, Fleetham JA. Natural head posture, upper airway morphology and obstructive sleep apnoea severity in adults. *Eur J Orthod*. 1998;20(2):133–143. doi:10.1093/ejo/20.2.133
10. DaCosta OO, Aikins EA, Isiekwe GI, Adediran VE. Malocclusion and early orthodontic treatment requirements in the mixed dentitions of a population of Nigerian children. *J Orthod Sci*. 2016;5(3):81–86. doi:10.4103/2278-0203.186164
11. Entrenas I, González-Chamorro E, Álvarez-Abad C, Muriel J, Menéndez-Díaz I, Cobo T. Evaluation of changes in the upper airway after Twin Block treatment in patients with Class II malocclusion. *Clin Exp Dent Res*. 2019;5(3):259–268. doi:10.1002/cre2.180
12. Verma G, Tandon P, Nagar A, Singh GP, Singh A. Cephalometric evaluation of hyoid bone position and pharyngeal spaces following treatment with Twin block appliance. *J Orthod Sci*. 2012;1(3):77–82. doi:10.4103/2278-0203.103863
13. O'Brien K, Wright J, Conboy F, et al. Effectiveness of early orthodontic treatment with the Twin-block appliance: A multicenter, randomized, controlled trial. Part 1: Dental and skeletal effects. *Am J Orthod Dentofacial Orthop*. 2003;124(3):234–243. doi:10.1016/S0889540603003524
14. Kinzinger G, Czapka K, Ludwig B, Glasl B, Gross U, Lisson J. Effects of fixed appliances in correcting Angle Class II on the depth of the posterior airway space: FMA vs. Herbst appliance – a retrospective cephalometric study. *J Orofac Orthop*. 2011;72(4):301–320. doi:10.1007/s00056-011-0035-2
15. Ahmadian-Babaki F, Araghbidi-Kashani SM, Mokhtari S. A cephalometric comparison of twin block and bionator appliances in treatment of Class II malocclusion. *J Clin Exp Dent*. 2017;9(1):e107–e111. doi:10.4317/jced.53031
16. Toth LR, McNamara JA Jr. Treatment effects produced by the twin-block appliance and the FR-2 appliance of Fränkel compared with an untreated Class II sample. *Am J Orthod Dentofacial Orthop*. 1999;116(6):597–609. doi:10.1016/s0889-5406(99)70193-9
17. Parkin NA, McKeown HF, Sandler PJ. Comparison of 2 modifications of the twin-block appliance in matched Class II samples. *Am J Orthod Dentofacial Orthop*. 2001;119(6):572–577. doi:10.1067/mod.2001.113790
18. LaHaye MB, Buschang PH, Wick Alexander RG, Boley JC. Orthodontic treatment changes of chin position in Class II Division 1 patients. *Am J Orthod Dentofacial Orthop*. 2006;130(6):732–741. doi:10.1016/j.ajodo.2005.02.028
19. Zhang C, He H, Ngan P. Effects of twin block appliance on obstructive sleep apnea in children: A preliminary study. *Sleep Breath*. 2013;17(4):1309–1314. doi:10.1007/s11325-013-0840-5
20. Gu M, Savoldi F, Hägg U, McGrath CP, Wong RWK, Yang Y. Upper airway changes following functional treatment with the headgear Herbst or headgear Twin Block appliance assessed on lateral cephalograms and magnetic resonance imaging. *ScientificWorldJournal*. 2019;2019:1807257. doi:10.1155/2019/1807257
21. Buschang PH, Tanguay R, Turkewicz J, Demirjian A, La Palme L. A polynomial approach to craniofacial growth: Description and comparison of adolescent males with normal occlusion and those with untreated Class II malocclusion. *Am J Orthod Dentofacial Orthop*. 1986;90(5):437–442. doi:10.1016/0889-5406(86)90009-0
22. Bishara SE, Jakobsen JR, Vorhies B, Bayati P. Changes in dentofacial structures in untreated Class II division 1 and normal subjects: A longitudinal study. *Angle Orthod*. 1997;67(1):55–66. doi:10.1043/0003-3219(1997)067<0055:CIDSIU>2.3.CO;2
23. Björk A, Skieller V. Facial development and tooth eruption. An implant study at the age of puberty. *Am J Orthod*. 1972;62(4):339–383. doi:10.1016/s0002-9416(72)90277-1
24. Vinoth SK, Thomas AV, Nethravathy R. Cephalomteric changes in airway dimensions with twin block therapy in growing Class II patients. *J Pharm Bioallied Sci*. 2013;5(Suppl 1):S25–S29. doi:10.4103/0975-7406.113288
25. Rose EC, Staats R, Lehner M, Jonas IE. Cephalometric analysis in patients with obstructive sleep apnea. Part I: Diagnostic value. *J Orofac Orthop*. 2002;63(2):143–153. doi:10.1007/s00056-002-0057-x
26. Ajami S, Morovvat A, Khademi B, Jafarpour D, Babanouri N. Dentoskeletal effects of Class II malocclusion treatment with the modified Twin Block appliance. *J Clin Exp Dent*. 2019;11(12):e1093–e1098. doi:10.4317/jced.56241
27. Almeida-Pedrin RR, Almeida MR, Almeida RR, Pinzan A, Carvalho Ferreira FP. Treatment effects of headgear biteplane and bionator appliances. *Am J Orthod Dentofacial Orthop*. 2007;132(2):191–198. doi:10.1016/j.ajodo.2005.07.030
28. Seifi M, Bargrizan M, Memar-Kermani N, Vahid-Dastjerdi E. The skeletal and alveolodental effects of an innovated functional appliance (Seifi functional – phase 1). *Iran J Orthod*. 2007;1(2):6–12. https://www.ijorth.com/article_247996.html. Accessed April 1, 2021.
29. Jamilian A, Showkatbakhsh R, Amiri SS. Treatment effects of the R-appliance and twin block in Class II division 1 malocclusion. *Eur J Orthod*. 2011;33(4):354–358. doi:10.1093/ejo/cjq082
30. Jena AK, Singh SP, Utreja AK. Effectiveness of twin-block and Mandibular Protraction Appliance-IV in the improvement of pharyngeal airway passage dimensions in Class II malocclusion subjects with a retrognathic mandible. *Angle Orthod*. 2013;83(4):728–734. doi:10.2319/083112-702.1
31. Fastuca R, Zecca PA, Caprioglio A. Role of mandibular displacement and airway size in improving breathing after rapid maxillary expansion. *Prog Orthod*. 2014;15(1):40. doi:10.1186/s40510-014-0040-2
32. Ghodke S, Utreja AK, Singh SP, Jena AK. Effects of twin-block appliance on the anatomy of pharyngeal airway passage (PAP) in class II malocclusion subjects. *Prog Orthod*. 2014;15(1):68. doi:10.1186/s40510-014-0068-3
33. Thapa VB, Shrestha A, Sherchan P, Poudel P, Joshi L. Twin block appliance: Effect on pharyngeal airway. *J. Kathmandu Med Coll*. 2018;7(4):147–152. doi:10.3126/jkmc.v7i4.23299
34. Yassaei S, Tabatabaei Z, Ghafurifard R. Stability of pharyngeal airway dimensions: Tongue and hyoid changes after treatment with a functional appliance. *Int J Orthod Milwaukee*. 2012;23(1):9–15. PMID:22533023.
35. Godt A, Koos B, Hagen H, Göz G. Changes in upper airway width associated with Class II treatments (headgear vs activator) and different growth patterns. *Angle Orthod*. 2011;81(3):440-446. doi:10.2319/090710-525.1

Original paper

The mRNA expression of genes encoding selected antioxidant enzymes and thioredoxin, and the concentrations of their protein products in gingival crevicular fluid and saliva during periodontitis

Joanna Toczewska[1,B–D], Dagmara Baczyńska[2,A,B,D], Anna Zalewska[3,A,E,F], Mateusz Maciejczyk[4,C,E,F], Tomasz Konopka[1,A–D]

[1] Department of Periodontology, Wroclaw Medical University, Poland
[2] Department of Molecular and Cellular Biology, Wrocław Medical University, Poland
[3] Experimental Dentistry Laboratory, Medical University of Bialystok, Poland
[4] Department of Hygiene, Epidemiology and Ergonomics, Medical University of Bialystok, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):255–265

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

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on January 6, 2022
Reviewed on April 3, 2022
Accepted on June 13, 2022

Published online on June 30, 2023

Cite as
Toczewska J, Baczyńska D, Zalewska A, Maciejczyk M, Konopka T. The mRNA expression of genes encoding selected antioxidant enzymes and thioredoxin, and the concentrations of their protein products in gingival crevicular fluid and saliva during periodontitis. *Dent Med Probl.* 2023;60(2):255–265. doi:10.17219/dmp/150888

DOI
10.17219/dmp/150888

Abstract

Background. The activity of antioxidant enzymes in periodontitis is reduced, but results vary between studies and are subject to bias. In turn, the expression of genes encoding antioxidant factors has not been examined yet.

Objectives. This is the first study to evaluate the expression of genes encoding superoxide dismutase 1 (SOD1), glutathione peroxidase 1 (GPX1) and thioredoxin 1 (TXN1) in the saliva and gingival tissue of patients with periodontitis. The activity of the antioxidant enzyme protein products in the unstimulated and stimulated saliva and the gingival crevicular fluid (GCF) of patients with periodontitis was also investigated.

Material and methods. The prospective study involved 65 patients with periodontitis, who were divided into groups depending on the disease stage, and a control group of 31 age- and gender-matched healthy patients.

Results. We demonstrated that the expression of genes encoding GPX1 and TXN1 in saliva was significantly higher, and the expression of genes encoding SOD1, GPX1 and TXN1 in the gingival tissue was significantly lower in periodontitis patients as compared to the control group. We noted a lower activity of GPX1 in unstimulated saliva, of SOD1 in stimulated saliva and of both antioxidant enzymes in GCF in patients with periodontitis.

Conclusions. The *GPX1* transcriptome and its activity in the salivary and GCF proteome appear to be dependent on the oxidative stress related to the destructive inflammatory changes in periodontitis.

Keywords: gene expression, saliva, periodontal disease, gingival crevicular fluid, salivary diagnostics

Introduction

During the course of periodontitis, due to the presence of a dysbiotic bacterial biofilm on the surface of dentin and cementum in the periodontal pocket, molecular pathways are activated in immune-inflammatory responses, leading to the destruction of the tooth-suspensory apparatus. Periodontitis is a social disease; in 2017, it was the 14th most common age-standardized diagnosis worldwide, with a prevalence of 9.8%, affecting approx. 796 million individuals worldwide.[1] Periodontitis is an independent risk factor for selected systemic diseases with significant mortality, including diabetes, metabolic syndrome and cardiovascular diseases.[2–4] Understanding the mechanisms of the protraction of inflammatory responses in periodontal tissues and the possibility of their effective interruption would be the basis for the primary prevention of the abovementioned diseases.

The periopathogen infection of the periodontal pocket stimulates a host immune-inflammatory response, in which neutrophils, T lymphocytes and B lymphocytes are activated. Neutrophils release reactive oxygen species (ROS) that directly damage periodontal tissues through lipid peroxidation, oxidative damage to proteins and DNA, the inhibition of cell growth, increased apoptosis, the destruction of the extracellular matrix (ECM) of gingival tissue and periodontal connective tissue, and elevated phosphatidylinositol activity.[5–7] Activated T and B lymphocytes induce the production of receptor activator for nuclear factor-kappa B ligand (RANKL), which is an osteoclastogenetic mediator for the appendicular bone. The ROS signaling leads to the activation of the mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) and nuclear transcription factor nuclear factor-kappa B (NFκB) pathways.[8] The NFκB signaling is the most important pro-transcription factor of numerous genes encoding pro-inflammatory cytokines, chemokines, adhesion molecules, and key enzymes for the synthesis of pro-inflammatory factors.[9,10] ROS detoxification is carried out by either cytoprotective antioxidant enzymes that prevent ROS from reacting with biological compounds (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and the thioredoxin (TXN) system) or non-enzymatic antioxidants that interrupt free radical reactions (among others, glutathione (GSH), ascorbate, tocopherol, uric acid, and coenzyme Q). The transcriptional regulators of antioxidant enzyme genes are FoxO proteins (SOD2, CAT, GPX) and sirtuins (SOD2, CAT).[8] Reactive oxygen species also activate cytoplasmic nuclear factor erythroid 2-related factor 2 (NRF2) through binding to Keap-1, resulting in its translocation to the nucleus and attachment to DNA, which initiates the transcription of genes that contain an antioxidant response element (ARE) sequence in the promoter. Those genes encode enzymes such as glutathione S-transferase, NADPH:quinone reductase, heme oxygenase, and γ-glutamylcysteine synthetase (γ-GCS).[11] Increasing NRF2 activity may be a therapeutic target in periodontitis, leading to elevated local antioxidant activity and reduced pro-inflammatory signaling.

The analysis of the activity of antioxidant enzymes throughout the course of periodontitis has led to conflicting observations with regard to gingival tissue and gingival crevicular fluid (GCF).[12–18] The evaluation of their activity in saliva previously indicated a significant decrease,[17,19,20] although some authors described a significant increase.[16,21] Those differences can be explained by the intensity of the inflammatory process, its duration, the variety of research methods, the influence of periopathogens, or genetic conditions. It would be interesting to relate the expression of selected gene transcripts encoding antioxidant factors in gingival tissue and saliva to the concentrations of their protein products in GCF and saliva.

The present study aimed to evaluate the expression of *SOD1*, *GPX1* and *TXN1* at the mRNA level in gingival tissue and saliva, together with the activity of SOD1 and GPX1 in GCF and saliva in the most advanced stages and grades of periodontitis. Moreover, we investigated the covariability of mRNA expression of these genes and the activity of both antioxidant enzymes with clinical exponents of periodontitis.

Material and methods

Patients

The study was conducted in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement. The protocol of the study was approved by the Bioethics Committee of Wroclaw Medical University (KB-559/2018). Informed written consent was obtained from all the subjects involved in the study.

During the study period (January–December 2019), 1,990 patients reported to the Department of Periodontology at Wroclaw Medical University, Poland, of which 1,635 had periodontitis in accordance with the guidelines established during the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions[22] (Fig. 1).

The exclusion criteria for both the study group and the control group were age below 20 and above 55 years, pregnancy, systemic comorbidities associated with oxidative stress (cancers, diabetes, hypertension, rheumatoid arthritis, kidney diseases, lung diseases, thyroid diseases), smoking in the 10 years preceding the study, the use of any medications or supplements in the 3 months preceding the study, the number of teeth below 15, the occurrence of clinical lesions in the oral cavity mucosa, or periodontal treatment less than a year before the study.

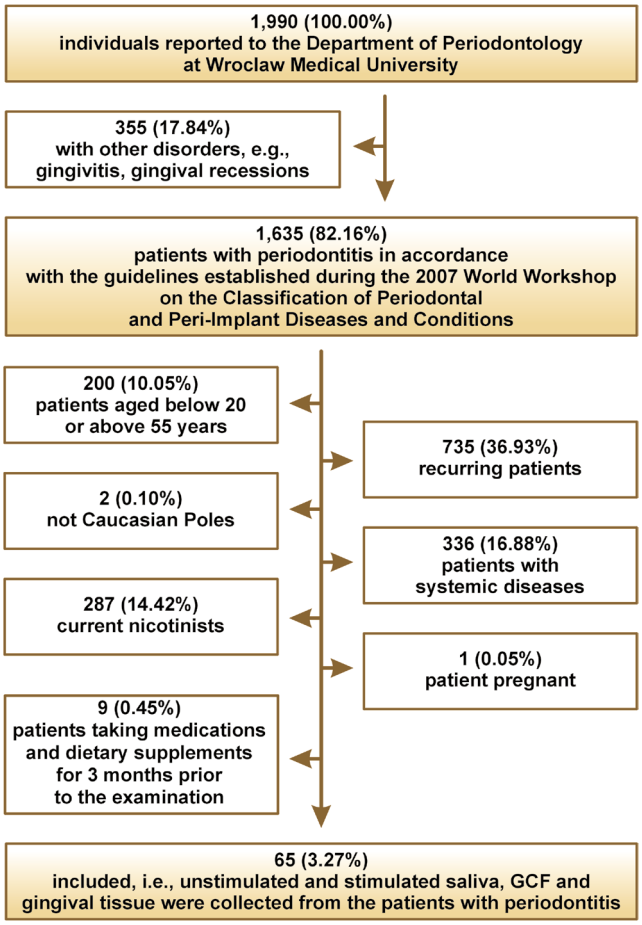

Fig. 1. Participant selection flow chart
GCF – gingival crevicular fluid.

Of the original patients, 65 (3.27%) were prospectively qualified for the study. People with periodontitis were divided into 2 subgroups based on the current clinical criteria, namely stage III or IV and stage B or C.[23]

The control group, selected by gender and age to match the study group, consisted of 31 generally healthy patients of the Academic Dental Polyclinic in Wroclaw, Poland, with clinically healthy periodontium (bleeding on probing (BOP) <10%, pocket depth (PD) ≤3 mm).

Unstimulated and stimulated saliva, GCF and gingival tissue were collected from all patients.

Saliva collection

To minimize the effect of the circadian rhythm on saliva secretion, the samples were collected between 8 a.m. and 10 a.m., with any additional stimuli eliminated. For 2 h before the examination, the patients refrained from oral hygiene procedures and the consumption of any food or beverages (excluding clean water). Saliva was collected in a sitting position, with the head slightly tilted downward, into a sterile Falcon® (DNA/RNA-free) tube placed in an ice container.[24,25] Saliva was collected using the spitting method after rinsing the patient's mouth 3 times with distilled water at room temperature and discarding the saliva collected during the 1st minute. Unstimulated saliva was collected for 10 min to a maximum volume of 5 mL. After a 5-minute break, stimulated saliva was collected for 5 min following stimulation by applying 10 µL of 2% citric acid on the tip of the tongue every 30 s. The volume of saliva was measured using an automated pipette, with an accuracy to 0.1 mL. Immediately after saliva was collected, the samples were centrifuged at 5,000 × g for 20 min at 4°C,[24,25] and the supernatant fluid, to which an antioxidant (10 µL of 0.5 M butylated hydroxytoluene per 1 mL of saliva) was added, was preserved for testing. Such samples were frozen at –80°C.[26] A 1-minute salivary flow, expressed in mL/min, was calculated by dividing the volume of saliva by the time necessary for its secretion.

GCF collection

The clinically deepest periodontal pockets were selected during the clinical examination. The region was isolated from saliva access with dental cotton rolls, and was then dried with compressed air. Gingival crevicular fluid was collected using PerioPaper Strips™, then an antioxidant (10 µL of 0.5 M butylated hydroxytoluene per 1 mL of GCF) was added and the samples were frozen at –80°C.[26] Any strips contaminated with blood or saliva were discarded. To determine the volume of GCF before and after the collection of the material, the strips were placed in Eppendorf® tubes and weighed on an analytical balance.[27,28]

Tissue collection

In the study group, gingival tissue was collected from the region of the deepest periodontal pocket at the time of periodontal treatment. The procedure was performed under topical anesthesia with articaine and adrenaline (Septanest, 1:100,000; Septodont, Saint-Maur-des-Fossés, France). According to the manufacturer's instructions, the material was placed in RNAlater® tubes and frozen at –80°C. Gingival fragments without signs of inflammation were collected from the control patients at the time of a third molar extraction. In all patients, tooth extraction was performed only for orthodontic reasons.

Clinical trial

Immediately after unstimulated saliva, stimulated saliva, GCF, and gingival tissue were collected, each patient had the dental examination performed by the same dentist (TK) according to the criteria of the World Health Organization (WHO), namely under artificial lighting, with the use of a mirror, an explorer and a periodontal probe.[29] The following clinical parameters were evaluated: the number of retained teeth; the mean mobility value of all teeth according to the indications of the Periotest® device;

the modified plaque index (PI) according to O'Leary et al.[17]; bleeding on probing (BOP) by Ainamo and Bay[18]; the papilla bleeding index (PBI) by Saxer and Mühlemann[19]; and the pocket depth (PD) measured at 6 points of each tooth. Furthermore, the mean PD for all teeth, the mean interproximal PD measured at 4 points of each tooth, the number of sites with PD > 5 mm, and the clinical attachment level (CAL) measured at 6 points of each tooth were calculated.

RNA isolation

The evaluation of the mRNA expression of the genes obtained from gingival tissue and saliva was performed at the Department of Molecular Techniques at Wroclaw Medical University.

The RNA isolation from the saliva samples was performed using the ISOLATE Biofluids RNA Kit (Bioline, London, UK) according to the manufacturer's instructions. Solid tissues were mechanically homogenized with the use of MagNA Lyser Green Beads (Roche Diagnostics, Mannheim, Germany) in Lysis Buffer and Homogenate Additive (Thermo Fisher Scientific, Inc., Waltham, USA). Subsequently, total RNA was extracted using the mirVana miRNA Isolation Kit (Thermo Fisher Scientific, Inc.), as recommended by the manufacturer. The RNA samples were then stored at –20°C.

RT-PCR

The cDNA synthesis was performed for each sample using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Inc.) with random hexamers and 10 µL or 14 µL of RNA isolated from solid tissues or saliva, respectively. Individual reactions were conducted in a total volume of 20 µL under the following thermal conditions: 25°C for 10 min; 37°C for 2 h; and 85°C for 5 min. The expression levels of *GPX1*, *SOD1* and *TXN* were measured by means of the relative real-time polymerase chain reaction (RT-PCR) method, using the TaqMan™ Gene Expression Assays (*GPX1*: Hs00829989_gH; *SOD1*: Hs00533490_m1; *TXN*: Hs00828652_m; *GAPDH*: Hs99999905_m1) and the TaqMan™ Fast Universal Master Mix (Thermo Fisher Scientific, Inc.). All reactions were performed in triplicate in a total volume of 10 µL, using the 7900HT Fast Real-Time PCR System (Thermo Fisher Scientific, Inc.), under the following thermal cycling conditions: 95°C for 20 s; 40 cycles of 95°C for 1 s; and 60°C for 20 s. All results were normalized against the expression of *GAPDH* and calculated using the $2^{-\Delta\Delta Ct}$ method.

Enzyme activity

The analysis of the activity of SOD1 and GPX1 in GCF and saliva was conducted in the Saliva Biochemistry Laboratory of the Department of Conservative Dentistry at the Medical University of Bialystok, Poland. All measurements were conducted using double tests and were standardized to milligram [mg] of total protein.

On the day of the measurements, the samples of saliva and GCF were slowly thawed at 4°C. To extract GCF, the PerioPaper Strips were placed in an Eppendorf test tube containing 0.02 M phosphate-buffered saline (PBS) solution with pH of 7.0 (1 strip/500 µL PBS). The samples were mixed for 30 s by using a vortex mixer, and then centrifuged at 3,000 × g for 5 min at 4°C. The supernatant fluid was preserved for testing.[27,28] An antioxidant (10 µL of 0.5 M butylated hydroxytoluene per 1 mL of GCF) was added to the samples containing GCF, and then they were mixed with a vortex mixer.[30] Gingival crevicular fluid was used for all measurements on the same day. The saliva and GCF samples were mixed with a vortex mixer immediately before the measurements.

The activity of SOD1 (E.C. 1.15.1.1) was measured by means of the colorimetric method described by Misra and Fridovich.[31] The principle of that method is based on the measurement of the cytoplasmic SOD activity subunit in the inhibition reaction of oxidation of epinephrine to adrenochrome at 320 nm. It was assumed that 1 unit of SOD activity inhibited 50% of epinephrine oxidation. The absorbance changes were measured at 320 nm. The SOD activity was measured in duplicate, and it was expressed in mU/mg of total protein.

The activity of GPX1 (E.C. 1.11.1.9) was evaluated colorimetrically, measuring the conversion of NADPH to NADP+ at 340 nm.[32] One unit of GPX activity was defined as the amount of enzyme that can catalyze the oxidation of 1 mmol NADPH per 1 min. The GPX1 activity was measured in duplicate, and it was expressed in mU/mg of total protein.

Statistical analysis

The Mann–Whitney *U* test was used for assessing differences between the 2 groups, whereas the evaluation of 3 groups was conducted using the Kruskal–Wallis test, followed by post hoc Tukey's test. Spearman's test was used for the correlation analysis. Statistical significance was determined at $p \leq 0.05$ for the Mann–Whitney and Kruskal–Wallis tests, while $p \leq 0.02$ was considered statistically significant for the correlation analysis. The statistical package Statistica, v. 13.3 (StatSoft, Cracow, Poland), was used.

Results

The general and periodontal data of the patients are shown in Table 1.

Table 1. General and periodontal data of the patients

Parameter		Control group			Stage III periodontitis group			Stage IV periodontitis group			All stages		
		Me	min	max	*Me*	min	max	*Me*	min	max	*Me*	min	max
Gender n (%)	M		14 (55)			19 (54)			14 (47)			33 (51)	
	F		17 (45)			16 (46)			16 (53)			32 (49)	
Age [years]		39	20	55	45	20	55	45	29	55	45	20	55
Unstimulated saliva flow [mL/min]		0.5	0.2	0.9	0.5	0.1	0.9	0.5	0.1	1.3	0.5	0.1	1.3
Stimulated saliva flow [mL/min]		1.5	0.4	3.4	1.4	0.3	3.0	1.6	0.6	3.5	1.5	0.3	3.5
Protein in unstimulated saliva [µg/mL]		659	301	1,101	821	481	1,387	840	24	1,847	827	24	1,847
Protein in stimulated saliva [µg/mL]		597	236	946	620	29	926	537	44	812	585	29	926
Protein in GCF [µg/mL]		29	8	92	131	37	337	134	46	446	131	37	446
Number of teeth		28	17	28	27	20	28	22	15	28	26	15	28
API		30	5	65	64	29	100	85	22	100	71	22	100
PI		20	0	81	46	7	100	43	0	100	43	0	100
BOP [%]		9	1	25	40	6	100	64	17	100	45	6	100
PD [mm]		1.5	1.3	2.1	3.1	2.1	5.3	4.1	2.7	5.4	3.5	2.1	5.4
CAL >0 [mm]		2.2	1.0	5.0	4.9	2.7	8.1	6.1	3.0	10.1	5.4	2.7	10.1

n – number; API – approximal plaque index; PI – plaque index; BOP – bleeding on probing; PD – pocket depth; CAL – clinical attachment level; M – male; F – female; *Me* – median; min – minimum; max –maximum.

mRNA data

The gingival *SOD1* mRNA expression was significantly higher in controls as compared to all patients with periodontitis. It was also significantly lower in both stages of periodontitis as compared to controls, although the difference in expression was not statistically significant in the most advanced stage of the disease. The salivary *SOD1* mRNA expression was not significantly different throughout the course of periodontitis and both its most advanced stages as compared to controls (Table 2).

The gingival *GPX1* mRNA expression was also significantly higher in controls as compared to all patients with periodontitis. It was significantly lower in stage III periodontitis as compared to controls. In contrast, the difference between stage IV periodontitis and controls was not statistically significant. The salivary *GPX1* mRNA expression was significantly higher throughout the course of periodontitis and both its most advanced stages as compared to controls, although it did not differ between the disease stages (Table 3).

Table 2. *SOD1* gene mRNA expression in gingival tissue and saliva depending on the stage of the disease and in the control group

Group	Material	*SOD1* expression	*p*-value
All stages (1)	gingival tissue	0.752 (0.425–1.660)	1 vs. 4 $p = 0.0061$*
Stage III (2)		0.752 (0.425–1.428)	2 vs. 3 vs. 4 $p = 0.0222$*
Stage IV (3)		0.761 (0.476–1.660)	2 vs. 4 $p = 0.0492$*
Control (4)		0.879 (0.588–2.017)	3 vs. 4 $p = 0.0960$ 2 vs. 3 $p = 1.0000$
All stages (1)	saliva	1.217 (0.295–4.290)	
Stage III (2)		1.264 (0.657–2.878)	1 vs. 4 $p = 0.1140$
Stage IV (3)		1.127 (0.295–4.290)	2 vs. 3 vs. 4 $p = 0.0690$
Control (4)		0.997 (0.483–1.758)	

Data presented as *Me* (min–max). * statistically significant.

Table 3. *GPX1* gene mRNA expression in gingival tissue and saliva depending on the stage of the disease and in the control group

Group	Material	*GPX1* expression	*p*-value
All stages (1)	gingival tissue	0.681 (0.064–2.756)	1 vs. 4 $p = 0.0132$*
Stage III (2)		0.492 (0.076–2.756)	2 vs. 3 vs. 4 $p = 0.0008$*
Stage IV (3)		0.906 (0.064–2.312)	2 vs. 4 $p = 0.0162$*
Control (4)		1.007 (0.421–2.271)	3 vs. 4 $p = 0.9200$ 2 vs. 3 $p = 0.0522$
All stages (1)	saliva	1.553 (0.311–8.670)	1 vs. 4 $p < 0.0001$*
Stage III (2)		1.846 (0.311–4.344)	2 vs. 3 vs. 4 $p = 0.0002$*
Stage IV (3)		1.539 (0.618–8.670)	2 vs. 4 $p = 0.0084$*
Control (4)		1.027 (0.519–1.730)	3 vs. 4 $p = 0.0092$* 2 vs. 3 $p = 0.9700$

Data presented as *Me* (min–max). * statistically significant.

The gingival *TXN1* mRNA expression was significantly higher in controls as compared to all patients with periodontitis. It was also significantly lower in both stages of periodontitis as compared to controls. The salivary *TXN1* mRNA expression was significantly higher during periodontitis progression and both its most advanced stages as compared to controls, although it did not differ between the disease stages (Table 4).

Table 4. *TXN1* gene mRNA expression in gingival tissue and saliva depending on the stage of the disease and in the control group

Group	Material	*TXN1* expression	*p*-value
All stages (1)	gingival tissue	0.391 (0.121–1.214)	1 vs. 4 $p < 0.0001$*
Stage III (2)		0.362 (0.121–1.180)	2 vs. 3 vs. 4 $p < 0.0001$*
Stage IV (3)		0.467 (0.158–1.214)	2 vs. 4 $p < 0.0001$*
Control (4)		0.969 (0.613–1.936)	3 vs. 4 $p < 0.0001$*
			2 vs. 3 $p = 0.6400$
All stages (1)	saliva	1.835 (0.700–3.497)	1 vs. 4 $p < 0.0001$*
Stage III (2)		1.794 (0.700–3.497)	2 vs. 3 vs. 4 $p < 0.0001$*
Stage IV (3)		1.863 (0.993–2.800)	2 vs. 4 $p < 0.0001$*
Control (4)		0.997 (0.483–1.758)	3 vs. 4 $p < 0.0001$*
			2 vs. 3 $p = 0.8900$

Data presented as *Me* (min–max). * statistically significant.

Enzyme activity

The activity of SOD1 in GCF was significantly lower in all patients with periodontitis as compared to controls. That observation was also true for stage IV periodontitis. Similarly, the SOD1 activity in unstimulated saliva was significantly lower in all study groups and in the patients with stage III periodontitis as compared to controls. There were even more clear differences in the SOD1 activity in stimulated saliva between the control group and the whole study group, as well as stage III and stage IV periodontitis (Table 5).

The activity of GPX1 in GCF was significantly lower in all patients with periodontitis as compared to controls, as well as in stage III and IV periodontitis. The GPX1 activity in unstimulated saliva was also significantly lower in all patients with periodontitis as compared to controls, and in stage III and IV periodontitis. The activity of GPX1 in stimulated saliva in all patients with periodontitis and its III and IV stages were, in turn, significantly higher as compared to controls (Table 6).

Clinical correlations

The analysis of the differences in the mRNA expression of the analyzed antioxidant genes, and in the activity of SOD1 and GPX1 in GCF and saliva between periodontitis with rapid and moderate progression rates demonstrated significantly higher SOD1 activity in stimulated saliva in grade C (Table 7).

The analysis of the covariation of the mRNA expression of the 3 analyzed genes between gingival tissue and saliva revealed no significant relationships. The same

Table 5. Comparison of the activity of superoxide dismutase 1 (SOD1) in saliva and gingival crevicular fluid (GCF) between the patients and the control group

Group	Material	SOD1 activity	*p*-value
All stages (1)	unstimulated saliva	16.50 (4.59–49.79)	1 vs. 4 $p = 0.0188$*
Stage III (2)		13.53 (6.96–49.79)	2 vs. 3 vs. 4 $p = 0.0474$*
Stage IV (3)		18.90 (4.59–38.18)	2 vs. 4 $p = 0.0260$*
Control (4)		19.70 (11.4–68.50)	3 vs. 4 $p = 0.1300$
			2 vs. 3 $p = 0.9000$
All stages (1)	stimulated saliva	1.97 (1.18–4.03)	1 vs. 4 $p < 0.0001$*
Stage III (2)		1.93 (1.18–3.55)	2 vs. 3 vs. 4 $p = 0.0001$*
Stage IV (3)		2.10 (1.28–4.03)	2 vs. 4 $p < 0.0001$*
Control (4)		6.57 (2.54–10.22)	3 vs. 4 $p < 0.0001$*
			2 vs. 3 $p = 0.9800$
All stages (1)	GCF	31.05 (10.70–65.52)	1 vs. 4 $p = 0.0010$*
Stage III (2)		31.90 (10.70–65.52)	2 vs. 3 vs. 4 $p = 0.0080$*
Stage IV (3)		27.60 (10.82–54.10)	2 vs. 4 $p = 0.2700$
Control (4)		39.92 (5.25–50.21)	3 vs. 4 p =0.0020*
			2 vs. 3 $p = 0.1000$

Data presented as *Me* (min–max). * statistically significant.

Table 6. Comparison of the activity of glutathione peroxidase 1 (GPX1) in saliva and gingival crevicular fluid (GCF) between the patients and the control group

Group	Material	GPX1 activity	*p*-value
All stages (1)	unstimulated saliva	10.44 (2.67–30.81)	1 vs. 4 $p < 0.0001$*
Stage III (2)		10.17 (6.08–25.60)	2 vs. 3 vs. 4 $p < 0.0001$*
Stage IV (3)		11.71 (2.67–30.81)	2 vs. 4 $p < 0.0001$*
Control (4)		17.74 (11.40–36.01)	3 vs. 4 $p = 0.0050$*
			2 vs. 3 $p = 0.7300$
All stages (1)	stimulated saliva	58.35 (38.98–102.40)	1 vs. 4 $p < 0.0001$*
Stage III (2)		58.36 (38.98–102.40)	2 vs. 3 vs. 4 $p < 0.0001$*
Stage IV (3)		58.35 (44.11–83.63)	2 vs. 4 $p = 0.0001$*
Control (4)		21.44 (13.39–39.85)	3 vs. 4 $p = 0.0001$*
			2 vs. 3 $p = 0.9700$
All stages (1)	GCF	11.00 (3.37–42.21)	1 vs. 4 $p = 0.0029$*
Stage III (2)		10.68 (3.67–42.21)	2 vs. 3 vs. 4 $p = 0.0083$*
Stage IV (3)		11.27 (3.37–32.03)	2 vs. 4 $p = 0.0180$*
Control (4)		17.80 (7.17–45.83)	3 vs. 4 $p = 0.0060$*
			2 vs. 3 $p = 0.7700$

Data presented as *Me* (min–max). * statistically significant.

applied to the correlations between the mRNA expression of *SOD1* and *GPX1* and the levels of their SOD1 and GPX1 products in GCF and both types of saliva (data not shown). A significant positive correlation between the *SOD1* mRNA expression in gingival tissue and the SOD1 activity in stimulated saliva in periodontitis patients with the fastest disease progression was the only exception ($R = 0.63$; $p = 0.002$).

The evaluation of the covariation of the *SOD1* mRNA expression as well as the SOD1 activity and clinical parameters in all patients with periodontitis showed no significant correlations. In the case of gingival *GPX1* mRNA expression, significant positive correlations were found with inflammation intensity (BOP: $R = 0.31$; $p = 0.019$) and the mean PD ($R = 0.37$; $p = 0.005$). The GPX1 activity in GCF and both types of saliva did not correlate with clinical parameters. The salivary *TXN1* mRNA expres-

Table 7. The mRNA expression of *SOD1*, *GPX1* and *TXN1* in gingival tissue and saliva, and the activity of superoxide dismutase 1 (SOD1) and glutathione peroxidase 1 (GPX1) in gingival crevicular fluid (GCF) and saliva depending on the progression of the disease

Antioxidant parameter	Stage	Material	*Me* (min–max)	*p*-value
mRNA *SOD1*	B	gingival tissue	0.80 (0.43–1.53)	0.970
	C		0.79 (0.46–1.66)	
mRNA *GPX1*	B	gingival tissue	0.78 (0.06–2.76)	0.400
	C		0.86 (0.13–2.76)	
mRNA *TXN1*	B	gingival tissue	0.44 (0.23–1.09)	0.390
	C		0.48 (0.12–1.21)	
SOD1	B	GCF	35.93 (10.70–65.52)	0.053
	C		28.18 (10.82–57.24)	
GPX1	B	GCF	13.91 (3.37–42.21)	0.490
	C		13.18 (3.67–32.03)	
mRNA *SOD1*	B	saliva	1.45 (0.61–4.29)	0.190
	C		1.29 (0.30–3.95)	
mRNA *GPX1*	B	saliva	1.79 (0.31–3.74)	0.760
	C		2.05 (0.62–8.67)	
mRNA *TXN1*	B	saliva	1.92 (0.70–3.50)	0.430
	C		1.99 (0.74–3.36)	
SOD1	B	unstimulated saliva	18.26 (4.59–44.93)	0.450
	C		21.08 (6.21–49.79)	
GPX1	B	unstimulated saliva	11.58 (2.67–30.81)	0.480
	C		13.56 (4.75–27.11)	
SOD1	B	stimulated saliva	1.88 (1.18–3.82)	0.020*
	C		2.28 (1.36–4.03)	
GPX1	B	stimulated saliva	58.59 (29.98–90.79)	0.330
	C		62.12 (32.06–102.40)	

* statistically significant.

sion displayed a significant correlation with the number of periodontal pockets >5 mm (R = 0.32; p = 0.013). In the most advanced stage of periodontitis, the following significant correlations between antioxidant and clinical parameters were observed: the salivary *SOD1* mRNA expression vs. BOP (R = 0.56; p = 0.006) and PBI (R = 0.51; p = 0.013); the salivary *GPX1* mRNA expression vs. PBI (R = 0.51; p = 0.019); the GPX1 activity in stimulated saliva vs. the percentage of teeth with CAL ≥ 5 mm on the interproximal surfaces (R = –0.61; p = 0.016); the gingival *TXN1* mRNA expression vs. the mean PD (R = –0.54; p = 0.007) and the number of periodontal pockets >5 mm (R = –0.57; p = 0.005); and the salivary *TXN1* mRNA expression vs. the number of sites with CAL > 5 mm (R = 0.54; p = 0.007). Only 4 statistically significant correlations were observed in patients with the fastest progression of periodontitis, namely the salivary *SOD1* mRNA expression vs. BOP (R = 0.53; p = 0.004) and PBI (R = 0.47; p = 0.013); the gingival *GPX1* mRNA expression vs. PBI (R = 0.46; p = 0.013); and the salivary *TXN1* mRNA expression vs. PI (R = 0.45; p = 0.013).

Discussion

The present study indicates that at the mRNA level of *SOD1* expression in the gingiva of patients with periodontitis, there is downregulation as compared to the clinically healthy periodontium. During the course of periodontitis (especially in its most advanced stage), the enzymatic activity of that protein was also reduced in GCF and both unstimulated and stimulated saliva. In the most rapidly progressive stage of periodontitis, a significant positive covariation was observed between *SOD1* mRNA expression in the gingiva and SOD1 activity in unstimulated saliva, as well as a significantly higher SOD1 activity in this type of saliva. Under the conditions of ROS and reactive nitrogen species (RNS) formation during periodontitis-related oxidative and nitrosative stress, there is an intranuclear downregulation of *SOD1* expression through the NRF2 signaling pathway.[33] The post-translational mechanisms of SOD1 regulation, namely phosphorylation, lysine modification and S-acetylation, also play an important role in its enzymatic activity.[34] The post-translational modification of histones through the epigenetic mechanisms, stimulated by periopathogens could also alter the expression of many genes, including antioxidant genes. However, one study showed no significant differences in the methylation of CpG sites of the *SOD1* gene in gingival epithelial cells between patients with periodontitis and controls.[35] The protraction of the inflammatory process in periodontal tissues leads to the enzymatic depletion of ROS scavengers, as shown by the evaluation of their activity, particularly in GCF.[36] In addition to the observations presented herein, a significant reduction of SOD1 activity in GCF during periodontitis has also been described by other authors.[17,18,37] Periopathogens play a special role in the processes taking place in the periodontal pockets. Sampath et al. demonstrated that *Porphyromonas gingivalis*-infected cells showed a significant elevation in the *GSK-3β*, and a reduction in *NRF* and *SOD1* mRNA expression as compared to uninfected cells.[38] Although the expression of the salivary mRNA transcript of *SOD1* did not differ between periodontally ill and healthy individuals in the present study, the salivary activity of that enzyme was markedly reduced during the course of periodontitis. The former part of the above observation has not been previously published, while the latter one is consistent with the results of other authors.[17,19,20] However, opposite results have also been reported.[16,21] These discrepancies are likely due to several determinants, namely the phase of periodontal tissue inflammation, its duration, the sources of salivary antioxidants other than those related to the periodontal pockets, the number and composition of bacteria in saliva, the short half-life of SOD1, the oral hygiene procedures, and methodological considerations.[39] The SOD1 activity in stimulated saliva was the only antioxidant parameter analyzed that stratified the degrees of periodontitis.

However, that was probably due to the outflow of blood from the periodontal pockets into saliva at the most advanced stage and grade of periodontitis, as indicated by significant positive correlations of salivary *SOD1* mRNA expression with the extent and intensity of the inflammatory response.

While the *SOD1* transcription appears to be independent of the direct influence of periodontitis, the *GPX1* RNA transcription may depend on the intensity of the destructive inflammatory process within periodontal tissues. In the present study, this is indicated by the significant positive correlations of the *GPX1* transcript levels with the intensity of gingival inflammation and periodontal pocket depths, as well as of the expression of this transcript in stage C disease with the inflammation intensity in the gingiva in all periodontitis patients. The expression of *GPX1* depends not only on ROS, but also on selenium availability, an inflammatory response to antigen stimulation and insulin resistance.[40] Through kinase phosphorylation pathways (c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK), MAPK, and others), ROS activate FoxO proteins, while elevated nuclear levels of FoxA1 are a factor that regulates the transcription of the *GPX1* gene encoding the cytoplasmic GPX1.[41] In the present study, the gingival *GPX1* mRNA expression was significantly reduced in all patients with periodontitis and those suffering from stage III periodontitis as compared to controls. This is not consistent with the observation made by Duarte et al., who found a significantly higher gingival *GPX1* mRNA expression in 15 generally healthy patients with periodontitis as compared to 12 controls.[42] Those differences may be due to the greater severity of periodontitis in relation to the present study (our patients suffered from stage IV periodontitis and the difference in the expression of this transcript was blurred as compared to controls) and different study group sizes. The depletion of the efficiency of the enzymatic antioxidant mechanisms during the long-term course of periodontitis is also indicated by our observation of a significantly lower GPX1 activity in GCF as compared to controls. The literature references for that observation range from those finding no difference to those showing a significantly higher activity throughout the course of periodontitis.[43,44] However, our results are supported by a reference showing the levels of oxidized glutathione in GCF, indicating a significant reduction in the mean GSH levels in GCF from the periodontal pockets as compared to clinically healthy sites.[45,46] This may be compounded by the mechanism of scavenging the bactericidal hypochlorous acid through adjusting the biosynthesis of intracellular glutathione in *Porphyromonas gingivalis*-infected gingival epithelial cells toward a phenotype that promotes periopathogen survival in the periodontal pockets.[47] Our analysis showed that the salivary *GPX1* mRNA expression and the GPX1 activity in stimulated saliva in all patients with periodontitis and those suffering from the 2 most advanced stages of the disease were significantly elevated, whereas they were reduced in unstimulated saliva as compared to controls. This confirms the assumption that the source of salivary RNA is not only the outflow of fluid and blood from the periodontal pocket (the positive correlation between the *GPX1* mRNA expression and PBI in the most advanced stage of the disease), but also the 3 major salivary glands, minor salivary glands, serum transudate in the salivary glands, and the exfoliating cells of the parakeratotic epithelium of the oral cavity.[48] The expression of salivary RNA with a short half-life is further influenced by a diverse oral microbiome, with significant differences in the content and types of endo- and exoribonucleases. Certainly, this may limit the sensitivity and specificity of such studies, although a strong pro-inflammatory stimulus in the form of extensive periodontitis can significantly alter the salivary transcriptome. The stimulation of saliva secretion provides additional extra-periodontal antioxidant factors, hence there are differences in the activity of enzymatic antioxidants between the evaluated types of saliva.[49] However, in other studies, the evaluation of the GPX1 activity in saliva was again differential, with unstimulated saliva showing a significant reduction or elevation,[21,43,50,51] while stimulated saliva demonstrated a reduction.[19]

A pioneering element of the current study was the evaluation of the *TXN1* mRNA expression in the gingiva and saliva of patients with periodontitis. Unfortunately, due to insufficient material for the study, the levels of TXN1 in GCF and saliva were not determined. There was a significant reduction in the gingival TXN1 mRNA expression both in the whole group of periodontitis patients and separately for the 2 most advanced stages of the disease. Moreover, in stage IV periodontitis, there was a significant negative correlation of that expression with the mean PD and the number of periodontal pockets deeper than 5 mm, although this does not confirm the dependence of this expression on the periodontitis progression. Oxidative stress leads to the dissociation of the Keap-1–NRF2 complex and the entry of NRF2 into the cell nucleus, where it binds to the adenylate-uridylate-rich elements (AREs) of DNA in the promoter regions of *TXN1* on chromosome 9q31.3 and *TXNRD1* on chromosome 12q23-q24.1.[52] Also, in the case of this enzymatic antioxidant system, this process is successively reduced with the long-term course of periodontopathy. In addition to ROS and RNS, other factors that enhance the expression of these genes include ultraviolet (UV) radiation, retinoic acid, selenium availability (TRXD1 is a selenoreductase), and reoxygenation after hypoxia. In the GCF extracted from healthy periodontal sites, a protein containing TXN domains was confirmed among 199 identified proteins following albumin depletion.[53] Through the spectrometric method of the matrix-assisted laser desorption/ionization (MALDI) with a tandem time-of-flight (TOF) analyzer (MALDI-TOF), TXN1 was found in the GCF extracted from the periodontal pockets.[54] After the use of liquid chromatography with

tandem mass spectrometry (LC-MS), a higher expression was found in GCF from the periodontal pockets as compared to gingival clefts.[55] Herein, a significantly higher salivary *TXN1* mRNA expression was found in periodontitis patients and its most advanced stages as compared to controls. This is likely due not only to the secretion of that transcript into saliva from the periodontal pockets (positive covariations with the number of periodontal pockets deeper than 5 mm in all periodontitis patients and the number of periodontal pockets with CAL above 5 mm in stage IV periodontitis), but also the product of glandular tissue of major and minor salivary glands,[56] blood serum filtrate, and epithelial cell exfoliation. Thioredoxin was found with the use of a quadrupole mass spectrometer (QMS) and a TOF analyzer, while thioredoxin peroxidase was observed in whole saliva by using MALDI-TOF.[57] A study of the proteome of unstimulated whole saliva (UWS) with the use of LC-MS significantly more frequently found TXN1 in the whole saliva of periodontitis patients as compared to periodontally healthy individuals, and also observed a significantly higher intensity of its expression during periodontitis and its close functional interactivity with catalase.[58] Interestingly, Lee et al. observed significant negative correlations between increases in the bleeding extent/PD and the TXN1 levels in stimulated saliva in individuals with untreated periodontitis (who had a dental check-up frequency of less than once a year).[59] Such correlations were not observed in patients with regular dental check-ups.[59] This shows the possibility of depletion of the TXN antioxidant system with the disease progression, and it may also involve other antioxidant enzymes. In our patients, who had the rapidly progressive form of periodontitis, a positive covariation was observed between the salivary *TXN1* mRNA expression and an indicator of the presence of a bacterial biofilm visible on the tooth surfaces. However, other authors do not confirm this relationship for the *TXN1* expression in saliva.[58,59] Perhaps this is not a coincidental relationship, but caused by a specific potentiation of the activity of the TXN system against the oxidative stress induced by the periopathogen *Fusobacterium nuclaetum*, which is the most important element in supragingival biofilm formation.[60]

Conclusions

In conclusion, during periodontitis as defined by the current trends, there is a significant reduction in the mRNA tissue expression of the *SOD1*, *GPX1* and, to the greatest extent, *TXN1* genes, as well as a significant elevation in the salivary expression of transcripts of the *GPX1* and *TXN1* genes. Surprisingly, patients with stage IV periodontitis were more similar to healthy controls than patients with stage III. This may be due to the induction of the antioxidant defense mechanisms, which increase with disease progression. It is well known that the enhancement of the antioxidant barrier is the primary adaptive mechanism to prevent oxidative damage in the oral cavity. In the case of 2 transcripts of gingival oxidative genes, no correlation with the activity of their protein products in GCF and saliva was observed. The *GPX* mRNA expression and the GPX activity in GCF and saliva appear to be most dependent on the oxidative stress related to the destructive inflammatory changes in periodontitis. It does not appear that any of the examined antioxidant elements could act as a predictor of the degree of periodontitis.

Further studies of antioxidant parameters in patients with periodontitis may lead to the elucidation of prognostic and diagnostic factors, and create concepts of new therapeutic strategies.

Ethics approval and consent to participate

The protocol of the study was approved by the Bioethics Committee of Wroclaw Medical University (KB-559/2018).Informed written consent was obtained from all the subjects involved in the study.

Data availability

The article contains complete data used to support the findings of the present study.

Consent for publication

Not applicable.

ORCID iDs

Joanna Toczewska https://orcid.org/0000-0002-7906-639X
Dagmara Baczyńska https://orcid.org/0000-0001-6781-6758
Anna Zalewska https://orcid.org/0000-0003-4562-0951
Mateusz Maciejczyk https://orcid.org/0000-0001-5609-3187
Tomasz Konopka https://orcid.org/0000-0002-8808-2893

References

1. Bernabe E, Mercenes W, Hernandes CR, et al.; GBD 2017 Oral Disorders Collaborators. Global, regional, and national levels and trends in burden of oral conditions from 1990 to 2017: A systematic analysis for the Global Burden of Disease 2017 study. *J Dent Res*. 2020;99(4):362–373. doi:10.1177/0022034520908533
2. Liccardo D, Cannavo A, Spagnuolo G, et al. Periodontal disease: A risk factor for diabetes and cardiovascular disease. *Int J Mol Sci*. 2019;20(6):1414. doi:10.3390/ijms20061414
3. Gobin R, Tian D, Liu Q, Wang J. Periodontal diseases and the risk of metabolic syndrome: An updated systematic review and meta-analysis. *Front Endocrinol (Lausanne)*. 2020;11:336. doi:10.3389/fendo.2020.00336
4. Sanz M, Marco del Castillo AM, Jepsen S, et al. Periodontitis and cardiovascular diseases: Consensus report. *J Clin Periodontol*. 2020;47(3):268–288. doi:10.1111/jcpe.13189
5. Kesarwala AH, Krishna MC, Mitchell JB. Oxidative stress in oral diseases. *Oral Dis*. 2016;22(1):9–18. doi:10.1111/odi.12300
6. Sacks D, Baxter B, Campbell BC, et al. Multisociety Consensus Quality Improvement Revised Consensus Statement for Endovascular Therapy of Acute Ischemic Stroke. *Int J Stroke*. 2018;13(6):612–632. doi:10.1177/1747493018778713

7. Maciejczyk M, Pietrzykowska A, Zalewska A, Knaś M, Daniszewska I. The significance of matrix metalloproteinases in oral diseases. *Adv Clin Exp Med*. 2016;25(2):383–390. doi:10.17219/acem/30428
8. Kanzaki H, Wada S, Narimiya T, et al. Pathways that regulate ROS scavenging enzymes, and their role in defense against tissue destruction in periodontitis. *Front Physiol*. 2017;8:351. doi:10.3389/fphys.2017.00351
9. Lingappan K. NF-κB in oxidative stress. *Curr Opin Toxicol*. 2018;7:81–86. doi:10.1016/j.cotox.2017.11.002
10. Borys J, Maciejczyk M, Antonowicz B, Sidun J, Świderska M, Zalewska A. Free radical production, inflammation and apoptosis in patients treated with titanium mandibular fixations – an observational study. *Front Immunol*. 2019;10:2662. doi:10.3389/fimmu.2019.02662
11. Chiu AV, Al Saigh M, McCulloch CA, Glogauer M. The role of NrF2 in the regulation of periodontal health and disease. *J Dent Res*. 2017;96(9):975–983. doi:10.1177/0022034517715007
12. Panjamurthy K, Manoharan S, Ramachandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. *Cell Mol Biol Lett*. 2005;10(2):255–264. PMID:16010291.
13. Akalin FA, Toklu E, Renda N. Analysis of superoxide dismutase activity levels in gingiva and gingival crevicular fluid in patients with chronic periodontitis and periodontally healthy controls. *J Clin Periodontol*. 2005;32(3):238–243. doi:10.1111/j.1600-051X.2005.00669.x
14. Borges I Jr., Machado Moreira EA, Filho DW, de Oliveira TB, Spirelle da Silva MB, Fröde TS. Proinflammatory and oxidative stress markers in patients with periodontal disease. *Mediators Inflamm*. 2007;2007:45794. doi:10.1155/2007/45794
15. Tonguç MÖ, Öztürk O, Sütçü R, et al. The impact of smoking status on oxidant enzyme activity and malondialdehyde levels in chronic periodontitis. *J Periodontol*. 2011;82(9):1320–1328. doi:10.1902/jop.2011.100618
16. Wei D, Zhang XL, Wang YZ, Yang CX, Chen G. Lipid peroxidation levels, total oxidant status and superoxide dismutase in serum, saliva and gingival crevicular fluid in chronic periodontitis patients before and after periodontal therapy. *Aust Dent J*. 2010;55(1):70–78. doi:10.1111/j.1834-7819.2009.01123.x
17. Karim S, Pratibha PK, Kamath S, et al. Superoxide dismutase enzyme and thiol antioxidants in gingival crevicular fluid and saliva. *Dent Res J (Isfahan)*. 2012;9:266–272.
18. Ghallab NA, Hamdy E, Shaker OG. Malondialdehyde, superoxide dismutase and melatonin levels in gingival crevicular fluid of aggressive and chronic periodontitis patients. *Aust Dent J*. 2016;61(1):53–61. doi:10.1111/adj.12294
19. Canakci CF, Cicek Y, Yildirim A, Sezer U, Canakci V. Increased levels of 8-hydroxydeoxyguanosine and malondialdehyde and its relationship with antioxidant enzymes in saliva of periodontitis patients. *Eur J Dent*. 2009;3(2):100–106. PMID:19421389. PMCID:PMC2676068.
20. Trivedi S, Lal N, Ali Mahdi A, Singh B, Pandey S. Association of salivary lipid peroxidation levels, antioxidant enzymes, and chronic periodontitis. *Int J Periodont Restorative Dent*. 2015;35(2):e14–e19. doi:10.11607/prd.2079
21. Novakovic N, Todorovic T, Rakic M, et al. Salivary antioxidants as periodontal biomarkers in evaluation of tissue status and treatment outcome. *J Periodontal Res*. 2014;49(1):129–136. doi:10.1111/jre.12088
22. Papapanou PN, Sanz M, Buduneli N, et al. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. *J Periodontol*. 2018;89(Suppl 1):S173–S182. doi:10.1002/JPER.17-0721
23. 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
24. Morawska K, Maciejczyk M, Popławski Ł, Popławska-Kita A, Kretowski A, Zalewska A. Enhanced salivary and general oxidative stress in Hashimoto's thyroiditis women in euthyreosis. *J Clin Med*. 2020;9(7):2102. doi:10.3390/jcm9072102
25. Skutnik-Radziszewska A, Maciejczyk M, Flisiak I, et al. Enhanced inflammation and nitrosative stress in the saliva and plasma of patients with plaque psoriasis. *J Clin Med*. 2020;9(3):745. doi:10.3390/jcm9030745
26. Gerreth P, Maciejczyk M, Zalewska A, Gerreth K, Hojan K. Comprehensive evaluation of the oral health status, salivary gland function, and oxidative stress in the saliva of patients with subacute phase of stroke: A case–control study. *J Clin Med*. 2020;9(7):2252. doi:10.3390/jcm9072252
27. Toczewska J, Konopka T, Zalewska A, Maciejczyk M. Nitrosative stress biomarkers in the non-stimulated and stimulated saliva, as well as gingival crevicular fluid of patients with periodontitis: Review and clinical study. *Antioxidants (Basel)*. 2020;9(3):259. doi:10.3390/antiox9030259
28. Toczewska J, Maciejczyk M, Konopka T, Zalewska A. Total oxidant and antioxidant capacity of gingival crevicular fluid and saliva in patients with periodontitis: Review and clinical Study. *Antioxidants (Basel)*. 2020;9(5):450. doi:10.3390/antiox9050450
29. World Health Organization (WHO). Oral health surveys: Basic methods – 5th ed. 2013, 1.137. https://www.who.int/publications/i/item/9789241548649. Accessed January 2, 2022.
30. Maciejczyk M, Gerreth P, Zalewska A, Hojan K, Gerreth K. Salivary gland dysfunction in stroke patients is associated with increased protein glycoxidation and nitrosative stress. *Oxid Med Cell Longev*. 2020:2020:6619439. doi:10.1155/2020/6619439
31. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem*. 1972;247(1):3170–3175. PMID:4623845.
32. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*. 1967;70(1):158–169. PMID:6066618.
33. Sima C, Aboodi GM, Lakschevitz FS, Sun C, Goldberg MB, Glogauer M. Nuclear factor erythroid 2-related factor 2 down-regulation in oral neutrophils is associated with periodontal oxidative damage and severe chronic periodontitis. *Am J Pathol*. 2016;186(6):1417–1426. doi:10.1016/j.ajpath.2016.01.013
34. Banks CJ, Andersen JL. Mechanisms of SOD1 regulation by post-translational modifications. *Redox Biol*. 2019;26:101270. doi:10.1016/j.redox.2019.101270
35. Coêlho MC, Queiroz IC, Viana Filho JM, de Aquino SG, Persuhn DC, Paulo de Oliveira NF. miR-9-1 gene methylation and DNMT3B (rs2424913) polymorphism may contribute to periodontitis. *J Appl Oral Sci*. 2020;28:e20190583. doi:10.1590/1678-7757-2019-0583
36. Mortazavi H, Ghasemi A, Vatankhah MR. Comparison of salivary total antioxidant levels in male smokers and non-smokers according to their personality types. *Dent Med Probl*. 2020;57(2):145–148. doi:10.17219/dmp/114440
37. Narendra S, Das UK, Tripathy SK, Sahani NC. Superoxide dismutase, uric acid, total antioxidant status, and lipid peroxidation assay in chronic and aggressive periodontitis patients. *J Contemp Dent Pract*. 2018;19(7):874–880. PMID:30066694.
38. Sampath C, Okoro EU, Gipson MJ, Chukkapalli SS, Farmer-Dixon CM, Gangula PR. *Porphyromonas gingivalis* infection alters Nrf2-phase II enzymes and nitric oxide in primary human aortic endothelial cells. *J Periodontol*. 2021;92(7):54–65. doi:10.1002/JPER.20-0444
39. Zieniewska I, Maciejczyk M, Zalewska A. The effect of selected dental materials used in conservative dentistry, endodontics, surgery, and orthodontics as well as during the periodontal treatment on the redox balance in the oral cavity. *Int J Mol Sci*. 2020;21(24):9684. doi:10.3390/ijms21249684
40. Huang JQ, Zhou JC, Wu YY, Ren FZ, Lei XG. Role of glutathione peroxidase 1 in glucose and lipid metabolism-related diseases. *Free Radic Biol Med*. 2018;127:108–115. doi:10.1016/j.freeradbiomed.2018.05.077
41. Klotz LO, Sànchez-Ramos C, Prietto-Arroyo I, Urbànek P, Steinbrenner H, Monsalve M. Redox regulation of FoxO transcription factors. *Redox Biol*. 2015;6:51–72. doi:10.1016/j.redox.2015.06.019
42. Duarte PM, Napimoga MH, Fagnani EC, et al. The expression of antioxidant enzymes in the gingivae of type 2 diabetics with chronic periodontitis. *Arch Oral Biol*. 2012;57(2):161–168. doi:10.1016/j.archoralbio.2011.08.007
43. Hendek MK, Erdemir EO, Kisa U, Ozcan G. Effect of initial periodontal therapy on oxidative stress markers in gingival crevicular fluid, saliva, and serum in smokers and non-smokers with chronic periodontitis. *J Periodontol*. 2015;86(2):273–282. doi:10.1902/jop.2014.140338
44. Wei PF, Ho KY, Ho YP, Wu YM, Yang YH, Tsai CC. The investigation of glutathione peroxidase, lactoferrin, myeloperoxidase and interleukin-1beta in gingival crevicular fluid: Implications for oxidative stress in human periodontal diseases. *J Periodontal Res*. 2004;39(5):287–293. doi:10.1111/j.1600-0765.2004.00744.x
45. Chapple IL, Brock G, Eftimiadi C, Matthews JB. Glutathione in gingival crevicular fluid and its relation to local antioxidant capacity in periodontal health and disease. *Mol Pathol*. 2002;55(6):367–373. doi:10.1136/mp.55.6.367

46. Grant MM, Brock GR, Matthews JB, Chapple IL. Crevicular fluid glutathione levels in periodontitis and the effect of non-surgical therapy. *J Clin Periodontol*. 2010;37(1):17–23. doi:10.1111/j.1600-051X.2009.01504.x
47. Roberts JS, Atanasova KR, Lee J, et al. Opportunistic pathogen *Porphyromonas gingivalis* modulates danger signal ATP-mediated antibacterial NOX2 pathways in primary epithelial cells. *Front Cell Infect Microbiol*. 2017;7:291. doi:10.3389/fcimb.2017.00291
48. Park NJ, Li Y, Yu T, Brinkman BM, Wong DT. Characterization of RNA in saliva. *Clin Chem*. 2006;52(6):988–994. doi:10.1373/clinchem.2005.063206
49. Maciejczyk M, Zalewska A, Gerreth K. Salivary redox biomarkers in selected neurodegenerative diseases. *J Clin Med*. 2020;9(2):497. doi:10.3390/jcm9020497
50. Miricescu D, Totan A, Calenic B, et al. Salivary biomarkers: Relationship between oxidative stress and alveolar bone loss in chronic periodontitis. *Acta Odontol Scand*. 2014;72(1):42–47. doi:10.3109/00016357.2013.795659
51. Guentsch A, Preshaw PM, Bremer-Streck S, Klinger G, Glockmann E, Sigusch BW. Lipid peroxidation and antioxidant in saliva of periodontitis patients: Effect of smoking and periodontal treatment. *Clin Oral Investig*. 2008;12(4):345–352. doi:10.1007/s00784-008-0202-z
52. Hawkes HJK, Karlenius TC, Tonissen KF. Regulation of the human thioredoxin gene promoter and its key substrates: A study of functional and putative regulatory elements. *Biochim Biophys Acta*. 2014;1840(1):303–314. doi:10.1016/j.bbagen.2013.09.013
53. Carneiro LG, Venuleo C, Oppenheim FG, Salih E. Proteome data set of human gingival crevicular fluid from healthy periodontium sites by multidimensional protein separation and mass spectrometry. *J Periodontal Res*. 2012;47(2):248–262. doi:10.1111/j.1600-0765.2011.01429.x
54. Ngo LH, Veith PD, Chen YY, Chen D, Darby IB, Reynolds EC. Mass spectrometric analyses of peptides and proteins in human gingival crevicular fluid. *J Proteome Res*. 2010;9(4):1683–1693. doi:10.1021/pr900775s
55. Bostanci N, Heywood W, Mills K, Parkar M, Nibali L, Donos N. Application of label-free absolute quantitative proteomics in human gingival crevicular fluid by LC/MS E (gingival exudatome). *J Proteome Res*. 2010;9(5):2191–2199. doi:10.1021/pr900941z
56. Kurimoto C, Kawano S, Tsuji G, et al. Thioredoxin may exert a protective effect against tissue damage caused by oxidative stress in salivary glands of patients with Sjögren's syndrome. *J Rheumatol*. 2007;34(10):2035–2043. PMID:17896802.
57. Huang CM. Comparative proteomic analysis of human whole saliva. *Arch Oral Biol*. 2004;49(12):951–962. doi:10.1016/j.archoralbio.2004.06.003
58. Resende Hartenbach FA, Velasquez É, Nogueira FC, Domont GB, Ferreira E, Vieira Colombo AP. Proteomic analysis of whole saliva in chronic periodontitis. *J Proteomics*. 2020;213:103602. doi:10.1016/j.jprot.2019.103602
59. Lee CY, Choy CS, Lai YC, et al. A cross-sectional study of endogenous antioxidants and patterns of dental visits of periodontitis patients. *Int J Environ Res Public Health*. 2019;16(2):180. doi:10.3390/ijerph16020180
60. Steeves CH, Potrykus J, Barnett DA, Bearne SL. Oxidative stress response in the opportunistic oral pathogen *Fusobacterium nucleatum*. *Proteomics*. 2011;11(10):2027–2037. doi:10.1002/pmic.201000631

Original paper

Scanning electron microscopy study to evaluate and compare fibrin clot adhesion over the root surface treated with tetracycline, doxycycline and minocycline

Swatantrata Dey[1,A–E], Anwesh Reddy Nandigam[1,A,C–F], Anil Kumar Kancharla[1,A,C,E,F], Sheema Tasneem Mohammad[1,A,C,D,F], Shiva Shankar Gummaluri[2,A,C,D,F], Hemalatha Doppalapudi[1,B,C], Anjaneya Mahapatra[1,B,C], Samuel Padala[1,B,C]

[1] Department of Periodontics and Oral Implantology, Sree Sai Dental College and Research Institute, Srikakulam, India
[2] Department of Periodontology and Implantology, GITAM Dental College and Hospital, Visakhapatnam, India

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):267–272

Address for correspondence
Swatantrata Dey
E-mail: dona.ultimate@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
We are very thankful to the College of Technology of Osmania University, Hyderabad, India, for their help in the conduction of the scanning electron microscopy examination.

Received on February 16, 2022
Reviewed on April 14, 2022
Accepted on April 19, 2022

Published online on June 29, 2023

Cite as
Dey S, Reddy Nandigam A, Kumar Kancharla A, et al. Scanning electron microscopy study to evaluate and compare fibrin clot adhesion over the root surface treated with tetracycline, doxycycline and minocycline. *Dent Med Probl.* 2023;60(2):267–272. doi:10.17219/dmp/149278

DOI
10.17219/dmp/149278

Abstract

Background. Periodontitis is an inflammatory disease caused by a group of specific microorganisms that provoke the destruction of the periodontal ligament and the alveolar bone, along with pocket formation or recession, or both.

Objectives. The present study aimed to compare the efficacy of tetracycline, doxycycline and minocycline in improving fibrin clot adhesion over manually instrumented periodontally affected root surfaces with the use of scanning electron microscopy (SEM).

Material and methods. A total of 45 single-rooted extracted teeth were sectioned into 45 dentinal blocks and divided into 3 groups: tetracycline (group I); doxycycline (group II); and minocycline (group III). A drop of blood was added over the dentinal blocks, allowed to clot, and later rinsed with phosphate-buffered saline (PBS),1% formaldehyde, and 0.02% glycine. Then, the surfaces were post-fixed in 2.5% glutaraldehyde and dehydrated in a graded ethanol series of 30%, 50%, 75%, 90%, 95%, and 100%. Afterward, the samples were examined under a SEM to assess fibrin clot adhesion and the number of blood cells.

Results. Minocycline demonstrated better fibrin clot adhesion, followed by tetracycline and doxycycline. Statistical significance was observed at ×2,000 magnification ($p = 0.021$), whereas no significance was noted at ×5,000 magnification.

Conclusions. Dentinal blocks treated with minocycline had a better fibrin network and a greater number of entrapped erythrocytes, which is vital in the early wound-healing process leading to the formation of connective tissue attachment.

Keywords: tetracycline, fibrin clot, minocycline, root biomodification

Introduction

Periodontitis is an inflammatory disease caused by a group of specific microorganisms that provoke the destruction of the periodontal ligament and the alveolar bone, along with pocket formation or recession, or both.[1] This inflammatory process changes the balance of the periodontium, observed as a loss of connective tissue attachment and of the alveolar bone, and the altered position of the junctional epithelium.[2] The inflammatory changes cause the exposition of cementum, leading to the accumulation of plaque and calculus on the surface, demineralization, the loss of collagen fibers, and contamination with cytotoxins and endotoxins, which in turn results in a reduction in fibroblast growth and viability, hindering new attachment.[3] Therefore, the goal of periodontal treatment is to preserve tooth functionality by attaining proper periodontal regeneration, which is achieved by stable clot formation that aids in optimal periodontal healing.[2,4]

The bacterial endotoxins present over the root surface are removed with the use of mechanical and chemical methods, as the mechanical methods alone do not ensure complete removal, but produce a compact smear layer that inhibits proper periodontal regeneration.[5] Thus, root biomodifiers are used as an adjunct to help remove the smear layer and restore the biocompatibility of the root surface by enhancing the exposure of the underlying radicular collagen fibrils.[6] The presence of the exposed dentin and cementum collagen fibers helps form proper and stable fibrin attachment in the blood clot, preventing epithelial downgrowth and forming a temporary scaffold required for cell growth and mature collagen adhesion.[7,8]

The application of root biomodifiers helps promote periodontal regeneration, as demonstrated by numerous in vitro and in vivo studies. Hence, various root biomodification agents have been introduced to detoxify and decontaminate the root surface.[2] Materials such as tetracycline, doxycycline and minocycline show bacteriostatic activity and have been proven to be very effective against a wide range of organisms when tested in humans and animals; they also affect the regeneration process by improving attachment levels, promoting gingival fibroblast growth, increasing substantivity, and inhibiting parathyroid hormone bone resorption.[3]

The present study used scanning electron microscopy (SEM) to compare the efficacy of tetracycline, doxycycline and minocycline in improving fibrin clot adhesion on manually instrumented periodontally affected root surfaces.

Material and methods

The present study was conducted between February 2021 and July 2021 after obtaining clearance from the Institutional Ethics Committee at the Sree Sai Dental College and Research Institute, Srikakulam, India (SSDCERI/IRB/IEC/2020-21/408/8/2). Forty-five single-rooted extracted teeth were collected at the Department of Oral and Maxillofacial Surgery of the Sree Sai Dental College and Research Institute, Srikakulam, India. The extracted teeth had to fulfill the following inclusion and exclusion criteria:

– the inclusion criteria – single-rooted teeth with normal morphology, and the absence of restorations or dental caries;
– the exclusion criteria – teeth with dental caries, restorations, malformations, or fractures.

Study design

The teeth extracted due to periodontal disease (grade III mobility) were initially cleaned with distilled water and stored in a saline solution for 2 months before the commencement of the experiment. The extracted teeth were then root-planed with area-specific Gracey curettes (Hu-Friedy, Chicago, USA), using 50 apico-coronal strokes parallel to the long axis of the tooth to remove the contaminants and form a smear layer (Fig. 1A). Using a straight bur, two parallel fissures were made on the proximal surface at the cementoenamel junction (CEJ) and 4 mm apically to the 1st fissure. The teeth were then sectioned between the fissures into 2 parts (Fig. 1B) to form 4 mm × 4 mm × 1 mm dentinal blocks (Fig. 1C), which were stored in phosphate-buffered saline (PBS). The prepared dentinal blocks were then randomly divided into 3 groups containing 15 samples each (n = 15).

Group I dentinal blocks were treated using burnishing cotton pellets soaked in tetracycline with light pressure for 2 min (Fig. 2A), and a similar process was followed using doxycycline (group II) and minocycline (group III).

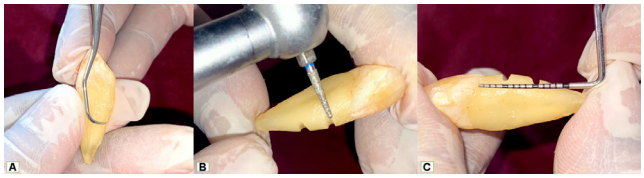

Fig. 1. Scaling and root planing (A), sectioning the tooth (B), and measuring the tooth (4 mm × 4 mm × 1 mm) (C)

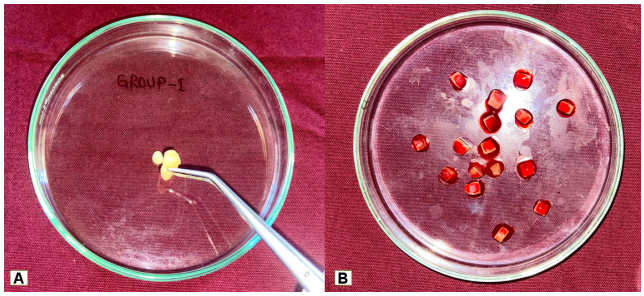

Fig. 2. Application of tetracycline (A) and a blood drop over the dentinal blocks for clot formation (B)

Preparation of tetracycline, doxycycline and minocycline solutions

One 500 mg tetracycline capsule (Resteclin 500; Abbott Healthcare, Mumbai, India), five 100 mg doxycycline capsules (Microdox-LBX; Micro Labs, Bangalore, India) and five 100 mg minocycline capsules (MINOZ™ 100; Sun Pharmaceutical Industries, Bangalore, India) were mixed with a saline solution (50 mL) in 3 separate glass beakers for 10 min by using a stirrer.

After applying tetracycline, doxycycline and minocycline, the samples were washed in 10 mL of saline solution. Blood was drawn from the cubital vein of a healthy volunteer with pristine gingiva and no systemic problems. A drop of blood was placed over the dentinal blocks and allowed to clot for 20 min (Fig. 2B).

Preparation of dentinal blocks for SEM

After clot formation, the samples were rinsed 3 times with PBS for 5 min in a Petri dish. After rinsing, the samples were fixed for 15 min in 1% formaldehyde diluted in PBS, followed by 3 rinses with PBS for 5 min each. The samples were incubated in 0.02 M glycine diluted in PBS, rinsed with PBS, post-fixed in 2.5% glutaraldehyde in PBS for 30 min, and rinsed again. The samples were washed and dehydrated in a graded ethanol series of 30%, 50%, 75%, 90%, and 95% for 10 min each, and then 3 times in 100% ethanol for 10 min. After this, the samples were stored in a desiccator jar until gold sputter coating was carried out. The dried specimens were mounted on the stubs of a scanning electron microscope and coated with gold and palladium in a sputter coating machine (Bal-Tec SCD 050; Bal-Tec, Los Angeles, USA). The samples were again stored in a desiccator jar at room temperature for 3 days,[9] and then examined under the SEM for the presence of a clot. The collected samples were analyzed using the HITACHI S-3700N SEM (Hitachi High-Tech, Hitachinaka, Japan) with a working distance (WD) of 13.3–16.4 mm, 15.0 kV, and magnification of ×2,000 and ×5,000.

The evaluation of fibrin clot adhesion and the number of blood cells was performed according to the blood elements adhesion index (BEAI) by Theodoro et al.[6,10] The scores were as follows: 0 for the absence of a fibrin network and blood cells; 1 for a scarcely distributed fibrin network and/or blood cells; 2 for a moderate number of blood cells and a thin fibrin network with poor interlacing; and 3 for a dense fibrin network with rich interlacing and the presence of blood cells.

Statistical analysis

The data was analyzed using IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA). Fisher's test compared the observed results with the expected results.

Results

The assessment of inter-examiner reliability employed Cohen's kappa statistic, and a κ-value of 0.721 indicated substantial agreement among the examiners. Kappa values indicate no agreement (≤0), none to slight (0.01–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), or almost perfect agreement (0.81-1.00).[11] To nullify the variation between examiners, we trained them and checked again for agreement. For the second test, the result produced a value of 0.82, which indicated almost perfect agreement.

Tetracycline group

×2,000 magnification

One sample from group I showed a dense fibrin network and blood cells, while 3 had a moderate fibrin network and blood cells. There was a scarcely distributed fibrin network and/or blood cells in 5 samples, whereas 6 treated samples had no fibrin network under this magnification (Table 1, Fig. 3A).

×5,000 magnification

One sample showed evidence of a dense fibrin network and blood cells, 2 had a moderate fibrin network and blood cells, 7 had a scarcely distributed fibrin network and/or blood cells, and 5 had no fibrin network under this magnification (Table 2, Fig. 3D).

Table 1. Comparison of the 3 groups at ×2,000 magnification (Fisher's exact test)

Group	Score 0	Score 1	Score 2	Score 3	*t*-value	*p*-value
Group I (*n* = 15)	6 (5.0)	5 (6.0)	3 (1.7)	1 (2.3)		
Group II (*n* = 15)	6 (5.0)	9 (6.0)	0 (1.7)	0 (2.3)	13.367	0.021*
Group III (*n* = 15)	3 (5.0)	4 (6.0)	2 (1.7)	6 (2.3)		

Groups: group I – tetracycline; group II – doxycycline; and group III – minocycline. * statistically significant.

Table 2. Comparison of the 3 groups at ×5,000 magnification (Fisher's exact test)

Group	Score 0	Score 1	Score 2	Score 3	*t*-value	*p*-value
Group I (*n* = 15)	5 (5.3)	7 (6.3)	2 (1.7)	1 (1.7)		
Group II (*n* = 15)	6 (5.3)	8 (6.3)	1 (1.7)	0 (1.7)	6.333	0.151
Group III (*n* = 15)	5 (5.3)	4 (6.3)	2 (1.7)	4 (1.7)		

Groups: group I – tetracycline; group II – doxycycline; and group III – minocycline.

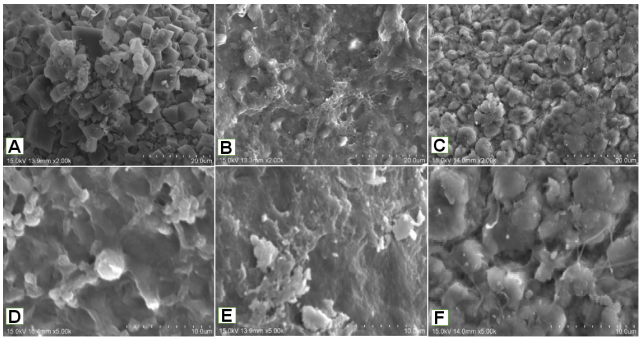

Fig. 3. Scanning electron microscopic (SEM) images

A–C – treated with tetracycline, doxycycline and minocycline, respectively (scores: A – 2; B – 0; C – 3) under ×2,000; D–F – treated with tetracycline, doxycycline and minocycline, respectively (scores: A – 1; B – 0; C – 3) under ×5,000 magnification.

Doxycycline group

×2,000 magnification

Nine samples from group II showed a scarcely distributed fibrin network and/or blood cells, whereas 6 had no evidence of fibrin network formation (Table 1, Fig. 3B).

×5,000 magnification

Eight samples from group II received a score of 1, 1 received a score of 2 and the rest of the root specimens did not appear to have any form of fibrin network (Table 2, Fig. 3E).

Minocycline group

×2,000 magnification

Six dentinal blocks from group III had a BEAI score of 3, and 2 samples received a score of 2. Only 4 samples seemed to have attained a scarcely distributed fibrin network, whereas 3 did not have any fibrin network (Table 1, Fig. 3C).

×5,000 magnification

Four samples in group III received a score of 3, which was attributed to the presence of a dense fibrin network and blood clots. Two samples received a score of 2, and 4 received a score of 1, while the rest of the root specimens did not show the presence of a fibrin network (Table 2, Fig. 3F).

Among the 3 groups, minocycline showed better fibrin clot adhesion and clot stabilization when compared to tetracycline and doxycycline at ×2,000 and ×5,000 magnification. Statistical significance was recorded at ×2,000 magnification (p = 0.021), whereas no significance was found at ×5,000 magnification (p = 0.151).

Discussion

The current study is the first to compare the efficacy of tetracycline, doxycycline and minocycline as root-conditioning agents for enhancing fibrin clot adhesion over the root surface. Hatfield and Baumhammers stated that when the root surface is exposed to mammalian cells and bacterial plaque, the cells show less affinity for cellular attachment unless mechanically debrided or cleaned.[12] Meanwhile, Aleo et al. stated that the primary attachment of connective tissue cells could be prevented by the presence of endotoxins that accumulate and get absorbed over the tooth surface, even after chemical debridement.[13] However, according to Genco and Mergenhagen, the host–microbe interaction is responsible for the destruction of the tooth and its supporting structures when affected with periodontitis.[14] The evaluation of fibrin clot adhesion over the root surface was considered a crucial factor in in vitro studies, as without proper root conditioning, clot formation is affected, leading to alterations in the tensile strength.[15]

In the present study, minocycline produced better results for fibrin clot adhesion among the 3 agents, as it favored periodontal wound healing and promoted periodontal regeneration. Tetracycline demonstrated a moderate effect, and doxycycline was the least effective.

Studies conducted by Somerman et al.[16] and Zhang et al.[17] suggested that treating the root surface with minocycline helped facilitate the adhesion of fibroblasts, as it has the potential to promote appropriate fibrin clot adhesion during periodontal surgery. Indeed, it enhanced periodontal healing, the growth of fibroblasts on the root surface and clinical attachment while promoting periodontal cell attachment. The minocycline ointment used has an optimum viscosity and a low pH, which helped to control the damaging effects at the application areas.[17]

Shetty et al. stated that minocycline had a consistent ability to eliminate the smear layer when compared to tetracycline, and also acts as a calcium chelator; its application resulted in enamel and root surface demineralization and the removal of endotoxins over the untreated root surfaces, which helped remove the smear layer and promoted binding with calcium phosphate, opening the collagen matrix.[18] Atilla and Baylas concluded in 1996 that applying 2.1% minocycline hydrochloride (HCl) ointment to the root surface binded calcium phosphate, and thus opened the collagen matrix, inhibiting the collagenase mechanism, and eliminated the smear layer caused by mechanical instrumentation.[19] Therefore 2.1% minocycline HCl ointment can be regarded as a potential root surface-conditioning agent.[19] Widaryono et al. showed that ethylenediaminetetraacetic acid (EDTA) and minocycline both had equal efficacy with regard to producing fibrin clots over the root surface; EDTA demonstrated the ability to eliminate the smear layer to open the cementum-collagen matrix, and also acted as a pH neutralizer, whereas minocycline removed the smear layer by itself.[20]

Tetracycline inhibits collagenase activity and bone resorption, and has antimicrobial activity during regeneration.[21] In its low-pH form, tetracycline can also increase the binding of fibronectin and other extracellular matrix (ECM) proteins to the root surface to enhance fibroblast attachment and growth, which in turn suppresses the proliferation and growth of epithelial cells.[22] According to studies conducted by Terranova et al.,[23] Bal et al.,[24] Larjava et al.,[25] and Steinberg and Willey,[26] using tetracycline as a root conditioner promoted optimal root surface characteristics for the binding of fibronectin, which causes the adhesion of fibrin to collagen, thus helping with fibroblast chemotaxis and binding, and leading to stable initial clot formation.[23,24] Furthermore, tetracycline improved clot organization and superficial demineralization, which was sufficient to attain the required exposure of collagen matrix, essential for proper clot adhesion.[25,26] In contrast, Delazari et al. stated that tetracycline HCl had no impact on the removal of the smear layer or fibrin network formation in the control and test groups.[27]

In the present study, the samples treated with tetracycline did not show the expected fibrin clot adhesion or presence of blood cells over the root surface, which may be due to the incomplete removal of the smear layer. Shetty et al. made a similar finding when reporting that minocycline had a better ability to remove the smear layer as compared to tetracycline.[18]

Doxycycline is an effective agent against different potentially causative microflora in periodontitis and has enzymatic properties. The topical application of doxycycline had a long-lasting substantivity on periodontally diseased root surfaces. The effect of doxycycline as a root-conditioning agent persists for around 14 days.[19]

A study conducted by Didhra et al. in 2020 used MTAD – a mixture of doxycycline (a tetracycline isomer), citric acid, polysorbate-80 (a detergent), and normal saline – and showed that it promoted better fibrin clot adhesion than saline.[4] Studies have used individual components of MTAD as periodontal conditioners, as low pH helps with the demineralization of the matrix and exposes collagen fibers to promote fibrin clot attachment. However, even with such persistent properties, in the present study, adequate fibrin clot adhesion was not observed for doxycycline, and better results were observed in the minocycline-treated group.

Conclusions

Within the limitations of the study, we concluded that although the 3 materials used belonged to the same group, only minocycline produced a biologically acceptable root surface and functioned as a biomodification agent. Indeed, minocycline helped form an extensive fibrin network with entrapped erythrocytes, which is vital in the early wound-healing process and leads to connective tissue attachment.

Ethics approval and consent to participate

The present study obtainined clearance from the Institutional Ethics Committee at the Sree Sai Dental College and Research Institute, Srikakulam, India (SSDCERI/IRB/IEC/2020-21/408/8/2).

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

Swatantrata Dey https://orcid.org/0000-0002-7666-2495
Anwesh Reddy Nandigam https://orcid.org/0000-0001-9321-4910
Anil Kumar Kancharla https://orcid.org/0000-0003-2161-0687
Sheema Tasneem Mohammad https://orcid.org/0000-0002-0044-8302
Shiva Shankar Gummaluri https://orcid.org/0000-0003-3892-7322
Hemalatha Doppalapudi https://orcid.org/0000-0002-0734-6018
Anjaneya Mahapatra https://orcid.org/0000-0002-0882-3015
Samuel Padala https://orcid.org/0000-0003-1034-2605

References

1. Grzech-Leśniak K, Matys J, Dominiak M. Comparison of the clinical and microbiological effects of antibiotic therapy in periodontal pockets following laser treatment: An in vivo study. *Adv Clin Exp Med*. 2018;27(9):1263–1270. doi:10.17219/acem/70413
2. Bhushan K, Chauhan, Praksh S. Root biomodification in periodontics – the changing concepts. *J Dent Oral Care Med*. 2016;2(1):105. doi:10.15744/2454-3276.2.105
3. Chahal GS, Chhina K, Chhabra V, Bhatnagar R, Chahal A. Effect of citric acid, tetracycline, and doxycycline on instrumented periodontally involved root surfaces: A SEM study. *J Indian Soc Periodontol*. 2014;18(1):32–37. doi:10.4103/0972-124X.128196
4. Didhra G, Upadhyay S, Sharma A, Sambyal H. Fibrin clot adhesion to instrumented conditioned root surfaces by MTAD and normal saline: A scanning electron microscopy study. *Arch Med Health Sci*. 2020;8(1):57–61. doi:10.4103/amhs.amhs_149_19
5. Kiryk J, Matys J, Grzech-Leśniak K, et al. SEM evaluation of tooth surface after a composite filling removal using Er:YAG laser, drills with and without curettes, and optional EDTA or NaOCl conditioning. Materials (Basel). 2021;14(16):4469. doi:10.3390/ma14164469
6. Nascimento GG, Leite AA, Manzolli Leite ER, Cezar Sampaio JE, Manzolli Leite FR. Blood clot stabilization on root dentin conditioned by the combination of tetracycline and EDTA. *Braz J Oral Sci*. 2014;13(2):83–88. doi:10.1590/1677-3225v13n2a01
7. Polimeni G, Xiropaidis AV, Wikesjö UM. Biology and principles of periodontal wound healing/regeneration. *Periodontol 2000*. 2006;41:30–47. doi:10.1111/j.1600-0757.2006.00157.x
8. Manzolli Leite FR, Cezar Sampaio JE, Zandim DL, Rached Dantas AA, Manzolli Leite ER, Leite AA. Influence of root-surface conditioning with acid and chelating agents on clot stabilization. *Quintessence Int*. 2010;41(4):341–349. PMID:20305869.
9. Rahed Dantas AA, Fontanari LA, de Paula Ishi E, et al. Blood cells attachment after root conditioning and PRP application: An in vitro study. *J Contemp Dent Pract*. 2012;13(3):332–338. doi:10.5005/jp-journals-10024-1147
10. Polson AM, Frederick GT, Ladenheim S, Hanes PJ. The production of a root surface smear layer by instrumentation and its removal by citric acid. *J Periodontol*. 1984;55(8):443–446. doi:10.1902/jop.1984.55.8.443

11. McHugh ML. Interrater reliability: The kappa statistic. *Biochem Med (Zagreb)*. 2012;22(3):276–282. PMID:23092060. PMCID:PMC3900052.
12. Hatfield CG, Baumhammers A. Cytotoxic effects of periodontally involved surfaces of human teeth. *Arch Oral Biol*. 1971;16(4):465–468. doi:10.1016/0003-9969(71)90170-1
13. Aleo JJ, De Renzis FA, Faber PA. In vitro attachment of human gingival fibroblasts to root surfaces. *J Periodontol*. 1975;46(11):639–645. doi:10.1902/jop.1975.46.11.639
14. Genco RJ, Mergenhagen SE. *Host-Parasite Interactions in Periodontal Diseases: Proceedings of a Symposium Held at Buffalo, New York, 4–6 May, 1981*. Washington, DC: American Society of Microbiology; 1982:235–245.
15. Preeja C, Janam P, Nayar BR. Fibrin clot adhesion to root surface treated with tetracycline hydrochloride and ethylenediaminetetraacetic acid: A scanning electron microscopic study. *Dent Res J (Isfahan)*. 2013;10(3):382–388. PMID:24019809. PMCID:PMC3760364.
16. Somerman MJ, Foster RA, Vorsteg GM, Progebin K, Wynn RL. Effects of minocycline on fibroblast attachment and spreading. *J Periodont Res*. 1988;23(2):154–159. doi:10.1111/j.1600-0765.1988.tb01349.x
17. Zhang H, Yang X, Li C, Shang S, Wang J. Effects of minocycline-HCl paste root conditioning on periodontal surgery: In vitro and in vivo studies. *Int J Clin Exp Med*. 2015;8(3):4080–4086. PMID:26064313. PMCID:PMC4443147.
18. Shetty B, Dinesh A, Seshan H. Comparitive effects of tetracyclines and citric acid on dentin root surface of periodontally involved human teeth: A scanning electron microscope study. *J Indian Soc Periodontol*. 2008;12(1):8–15. doi:10.4103/0972-124X.44090
19. Atilla G, Baylas H. Effect of various demineralizing agents on mineral contents of cementum surfaces (an electron probe analysis). *J Marmara Univ Dent Fac*. 1996;2(2–3):515–519. PMID:9569807.
20. Widaryono A, Soeroso S, Lelyati S. Effectivity of 2.1% minocycline ointment and 24% ethylenediaminetetraacetic acid gel as a root surface conditioning material (a study of fibrin clot adhesion with scanning electron microscope). *Int J App Pharm*. 2019;11(1):59–63. doi:10.22159/ijap.2019.v11s1.166
21. Hanes PJ, O'Brien NJ, Garnick JJ. A morphological comparison of radicular dentin following root planing and treatment with citric acid or tetracycline HCl. *J Clin Periodontol*. 1991;18(9):660–668. doi:10.1111/j.1600-051x.1991.tb00107.x
22. Madison JG 3rd, Hokett SD. The effects of different tetracyclines on the dentin root surface of instrumented, periodontally involved human teeth: A comparative scanning electron microscope study. *J Periodontol*. 1997;68(8):739–745. doi:10.1902/jop.1997.68.8.739
23. Terranova VP, Franzetti LC, Hic S, et al. A biochemical approach to periodontal regeneration: Tetracycline treatment of dentin promotes fibroblast adhesion and growth. *J Periodontal Res*. 1986;21(4):330–337. doi:10.1111/j.1600-0765.1986.tb01467.x
24. Bal B, Eren K, Balos K. Effects of various root surface treatments on initial clot formation: A scanning electron microscope study. *J Nihon Univ Sch Dent*. 1990;32(4):281–293. doi:10.2334/josnusd1959.32.281
25. Larjava H, Salonen J, Häkkinen L, Närhi T. Effect of citric acid treatment on the migration of epithelium on root surfaces in vitro. *J Periodontol*. 1988;59(2):95–99. doi:10.1902/jop.1988.59.2.95
26. Willey R, Steinberg AD. Scanning electron microscopic studies of root dentin surfaces treated with citric acid, elastase, hyaluronidase, pronase and collagenase. *J Periodontol*. 1984;55(10):592–596. doi:10.1902/jop.1984.55.10.592
27. Delazari FM, Gerlach RF, Joly JC, de Lima AF. Scanning electron microscopy study of the effect of tetracycline HCl on smear layer removal and fibrin network formation. *Braz Dent J*. 1999;10(2):81–87. PMID:10863393.

Original paper

Retromolar canal: Frequency in a Polish population based on CBCT and clinical implications – a preliminary study

Magdalena Piskórz[1,A,B,E], Aleksandra Bukiel[2,B–D], Karolina Kania[2,B–D], Dorota Kałkowska[2,B–D], Ingrid Różyło-Kalinowska[1,E,F]

[1] Department of Dentomaxillofacial Radiodiagnostics, Medical University of Lublin, Poland
[2] Student Research Group at the Department of Dentomaxillofacial Radiodiagnostics, Medical University of Lublin, Poland

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):273–278

Address for correspondence
Magdalena Piskórz
E-mail: magdalena.piskorz@umlub.pl

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on December 12, 2021
Reviewed on February 24, 2022
Accepted on February 28, 2022

Published online on June 29, 2023

Abstract

Background. A retromolar canal (RMC) is an anatomical variation of the mandibular canal located in the retromandibular area. Retromolar canals and their contents may be of great clinical importance for clinicians dealing with the discussed region. The analysis of the available literature indicates that RMC is not a rare phenomenon.

Objectives. The purpose of the present study was to present the prevalence of RMC and its dependence on patient gender, as well as the location of RMC (unilateral or bilateral), by using cone-beam computed tomography (CBCT).

Material and methods. Two hundred CBCT examinations taken from the database of the Department of Dental and Maxillofacial Radiodiagnostics of the Medical University of Lublin, Poland, were analyzed by 2 independent observers (a fifth-year dentistry student and a dentist with 9 years of experience in the field of dental and maxillofacial radiodiagnostics). The research sample included 134 women and 66 men.

Results. After comparing the results obtained by the 2 independent observers, the more experienced researcher excluded 9 cases from the study; RMC was ultimately found in 21/200 subjects (10.5%). The unilateral variant was observed in all 21 cases – 13/21 (61.9%) on the right side and 8/21 (38.1%) on the left side. Seven (5.2%) RMCs were found among the 134 women, while among the 66 men there were14 (21.2%) RMCs found.

Conclusions. On the basis of the conducted research, RMCs were found in 10.5% of cases. They were more common in men than in women. Cone-beam computed tomography is an examination that allows the determination of the position and course of RMC more precisely than panoramic X-rays.

Keywords: mandible, anatomy, CBCT, retromolar canal

Cite as
Piskórz M, Bukiel A, Kania K, Kałkowska D, Różyło-Kalinowska I. Retromolar canal: Frequency in a Polish population based on CBCT and clinical implications – a preliminary study. *Dent Med Probl.* 2023;60(2):273–278. doi:10.17219/dmp/147005

DOI
10.17219/dmp/147005

Introduction

A retromolar canal (RMC) and a retromolar foramen (RMF) are variations in the anatomical structure of the mandible. The canal is relatively short, deviates from the mandibular canal at different heights, and ends at the upper surface of the alveolar part of the mandible in the retromolar area as RMF.[1] The contents of the canal can consist of nerve fibers from the inferior alveolar nerve, as well as blood vessels.[2] According to Bilecenoglu et al., an artery of a 120–130 mm lumen is present in RMC.[3] After exiting the aperture, nerve fibers branch out. They can innervate the temporalis tendon, the buccinator muscle, the mandibular third molar, the gingiva in the area of mandibular premolars and molars, and the mucosa of the retromolar area.[4] The presence of RMC can be associated with complications during surgical procedures. It may also be a pathway for tumors or the spread of infections.[5] There are many different classifications that describe the anatomy of the canal. In 2011, Von Arx et al. proposed a classification, differentiating 3 types of canals.[6] In 2013, Patil et al. subdivided one of the types proposed by Von Arx et al. into 2 variants.[7]

The selected classification, according to Patil et al.,[7] describes 4 types of RMC alignment (self-drawn) (Fig. 1):

- type A – the canal branches vertically from the mandibular canal, distally from the third molar, it runs backward and upward, and then opens within the retromolar area:
  - type A1 – the canal runs backward and upward to open into the retromolar area,
  - type A2 – the canal runs a certain distance forward, and then reverses backward and upward to open into the retromolar area;
- type B – the canal branches below the mandibular foramen, and then runs forward and upward to open into the retromolar area; and
- type C – the canal separates near the mandibular foramen, and then runs horizontally forward to open within the retromolar area.

This research was based on evaluating cone-beam computed tomography (CBCT) images. It is a relatively new but already widespread modality in dental diagnostic imaging. Most CBCT units are similar in shape to panoramic models. Some manufacturers offer units that provide both types of imaging – panoramic and CBCT. During the examination, an X-ray tube emits a cone-shaped beam during the rotation of a C-shaped arm around the patient's head. The data on X-ray attenuation is recorded within a cylindrical volume. In the next stage, a primary reconstruction is prepared by computer software, and eventually, multiplanar reconstructions are created. Depending on the indications, the field of view (FOV) may vary from small through medium to large.

In addition to RMCs, there are other possible variations of anatomical anomalies, such as coronoid process hypertrophy (CPH), which is also defined as giant coronoid syndrome. Coronoid process hypertrophy consists of an abnormal increase in the volume of the mandibular process. One symptom of CPH that almost always occurs is decreased mouth opening (<20 mm). The etiology of CPH is still not conclusive. The treatment consists of intraoral coronoidectomy along with postoperative physiotherapy at the end of growth to obtain the correct range of jaw opening and to maintain it in the long term.[8] Another example of an anatomical abnormality is the orofaciodigital (OFD) syndrome. The disease manifests itself in a group of disorders, including the malformations of the mouth, face, hands, and feet. The OFD syndrome is inherited. For dentists, the most important symptoms are changes in the oral cavity. The teeth are often affected by caries, there are teeth with abnormal structures, there is enamel hypoplasia, supernumerary teeth can appear, and tooth agenesis can be observed.[9] A patient with a complex image must be treated in a multidisciplinary manner and requires prosthetic rehabilitation, in particular, in order to supplement the missing teeth in the arch.[10]

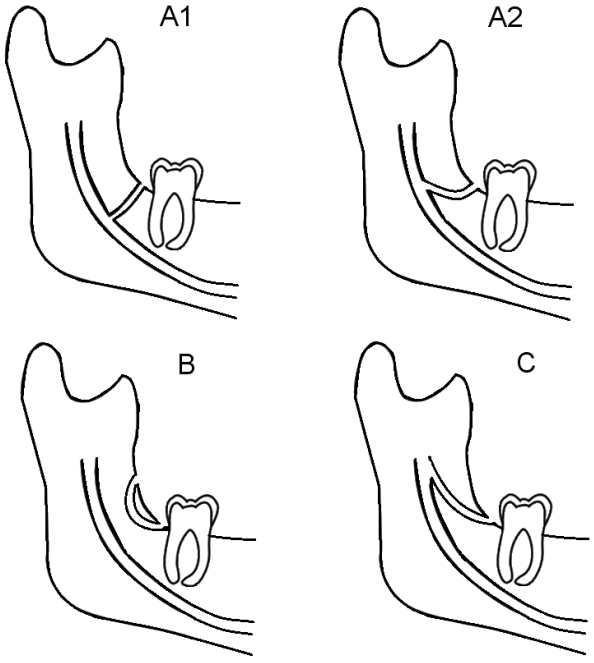

Fig. 1. Classification of the retromolar canal (RMC) according to Patil et al.[7] – own elaboration

The aim of the present study was to determine the prevalence of RMC and its dependence on patient gender, as well as the location of RMC (unilateral or bilateral), by using CBCT.

Material and methods

Two hundred consecutive CBCT examinations retrieved from the database of the Department of Dental and Maxillofacial Radiodiagnostics of the Medical University

of Lublin, Poland, were analyzed by 2 independent observers (a fifth-year dentistry student and a dentist with 9 years of experience working in the Department of Dental and Maxillofacial Radiodiagnostics). The examinations were taken with a VistaVox S CBCT (Dürr Dental, Bietigheim-Bissingen, Germany). The inclusion criteria were patients ≥18 years old and good-quality X-ray examinations in the retromolar area (i.e., without distortion and any artifacts caused by metal restorations). The exclusion criteria comprised bone with any pathologies, such as cysts, tumors, inflammation, etc. The research group included 134 women and 66 men aged 18–55 years, with an average age of 30.82 years. The CBCT scans were analyzed in multiplanar reconstructions (coronal, axial and sagittal), using modern ergonomic image processing software VistaSoft (Dürr Dental) and the Coronis Fusion 4MP radiological diagnostic display system (MDCC-4430; Barco, Kortrijk, Belgium). Additionally, on the basis of the tangential view, the type of RMC was determined according to the classification of Patil et al.[7]

Results

Initially, 30 RMCs were found in the 200 scans. After comparing the results of the 2 independent observers, the more experienced researcher excluded 9 cases from the study, so RMCs were ultimately found in 21/200 subjects (10.5%). In all 21 cases, the canals were unilateral; in 13/21 (61.9%) patients, the RMC was on the right side, and in 8/21 (38.1%) on the left side. Among the 134 women, an additional canal was found in 7 cases (5.2%), while among the 66 men, an additional canal was found in 14 (21.2%) cases.

The results showing the prevalence of the types of RMC according to Patil et al.'s classification[7] are presented in Table 1 and Fig. 2. The examples of each type are presented in Fig. 3.

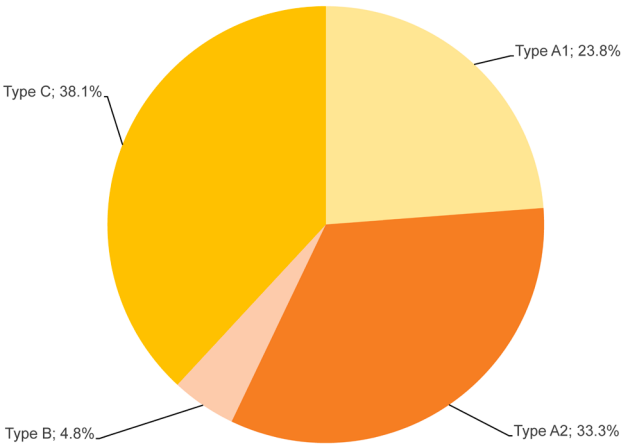

Fig. 2. Results showing the prevalence of the types of the retromolar canal (RMC) according to Patil et al.'s classification[7]

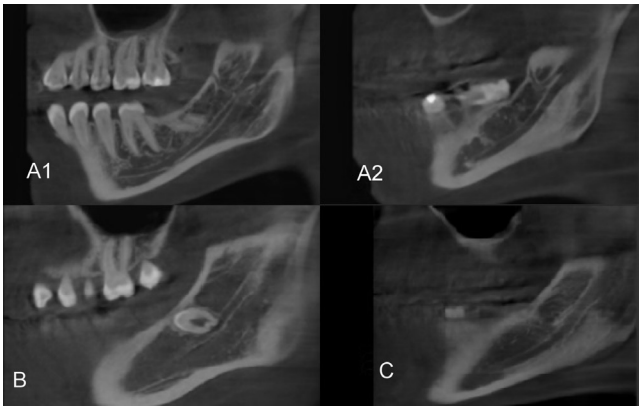

Fig. 3. Tangential views presenting the examples of the retromolar canal (RMC) types according to Patil et al.'s classification[7]

Table 1. Prevalence of the types of the retromolar canal (RMC) according to Patil et al.'s classification[7] with regard to gender

RMC type	Gender		Total
	M	F	
A1	4	1	5 (23.8)
A2	5	2	7 (33.3)
B	1	0	1 (4.8)
C	4	4	8 (38.1)
Total	14	7	21 (100)

Data presented as number (percentage) (*n* (%)). M – male; F – female.

Discussion

Numerous studies have used CBCT examinations to determine the prevalence of RMC,[1,2,4–6,11,12] but none of them were focused on a Polish population. This research was based on CBCT examinations and revealed the presence of RMC in 10.5% of cases. In other studies, the prevalence of RMC ranges from 5.4% to 75.4%.[4] Such a discrepancy can be attributed to several factors, including ethnic differences, environmental factors and genetic diversity, as well as the sample size of the study.

Ahmed et at. showed in their study that the incidence of RMC in CBCT images was 12%, with more identified on the left side (5%) than on the right side (7%).[13] Hou et al.[5] confirmed the presence of RMC in 25.9% of 657 patients.[5] Depending on gender, RMC was observed in 11.6% of men and 14.3% of women. Unilateral RMCs were identified in 20.4% of patients, while 5.5% of the RMCs were bilateral.[5] Von Arx et al. estimated the prevalence of RMC at 25.6%.[6] According to Hou et al., the canal was found in the range of 8.5–52% in a Chinese population.[5] In a Korean population, RMCs were observed in 8.5% of patients (38/446).[14]

In our study, the canal was always located unilaterally (61.9% on the right side and 38.1% on the left side), which is consistent with other studies that focused on the location of RMC.[4–6,15,16]

Taking gender into account, our results showed that RMC was more common in men. Out of the 66 examined men, 14 (21.2%) had RMC, while out of the 134 examined women, RMC was noted in 7 (5.2%) cases. A study conducted by Jamalpour et al. did not present a large difference in the occurrence of the canal depending on gender; they showed that RMCs were present in 10.3% of women and 16.7% of men.[17] Narayana et al. studied 242 dry adult mandibles in an Indian population.[18] Regarding gender, men tended to have RMC more often than women, but no statistically significant difference was found in this respect.[18]

Considering the types of RMC according to the classification of Patil et al.,[7] the most common type of RMC found in our research was type A (57.1%), of which 23.8% were A1 and 33.3% were A2, followed by type C (38.1%), and then type B (4.8%).

A significant problem raised in the literature about the classification of the canal. Some authors believe that it is one of the variants of double or triple canals that depart from the main canal and end in the molar area, while other authors believe that it constitutes a separate structure.[1]

Cone-beam computed tomography, computed tomography (CT) and panoramic radiographs are used to detect RMFs and RMCs.[4]

In a study by Kim et al., only 25% of panoramic radiographs showed mandibular canals.[2] Although panoramic radiograph images are frequently used for the preliminary planning of treatment, in some cases, it is recommended to extend the diagnostics by taking volumetric tomography. Cone-beam computed tomography more often visualizes the canal and makes it possible to trace its course, which is of great importance in clinical situations. The disadvantage of CBCT is related to the presence of artifacts, defined as discrepancies between the reconstructed visual image and the actual subject, which degrades the quality of these images.[4] It also emits ionizing radiation and has limited contrast resolution.[19] It is also possible to visualize RMCs on CT, but its use in dentistry is limited due to cost, availability, and the risk related to a higher radiation dose as compared to CBCT and X-rays.[12] Furthermore, CT artifacts can include noise, motion, beam hardening, scatter, and metal artifacts. Cone-beam computed tomography is the preferred method due to its lower radiation dose, lower costs and a better detection of anatomical structures.[4] It is safe and painless for patients of all ages. It is also better for analyzing the position and orientation of the surrounding structures.[19]

The ability to detect RMCs with panoramic radiography is limited. Cone-beam computed tomography can be useful in confirming the anatomical variations of the mandibular canal which cannot be visualized with panoramic radiographs. Panoramic radiography is less sensitive, lacks detail, and is characterized by irregular magnification, geometric distortion and the overlapping of anatomical structures. Another disadvantage includes ghost shadows, which are produced by the contralateral side of the mandible, the pharyngeal airway, the soft palate, and the uvula.[4]

In a study by Von Arx et al., out of the 31 canals observed with CBCT, only 7 were visible in the corresponding panoramic radiographs.[6] In a case report, Kaufmann et al. presented a bilateral RMC that was observed on CT, but was not visible with panoramic radiography.[20]

Based on the literature, it can be concluded that the clarity of CBCT images is affected by artifacts, noise and poor soft tissue contrast.[21] Magnetic resonance imaging (MRI) is the most appropriate modality for soft tissue diagnostics. In dentistry, MRI is particularly useful for diagnosing diseases of the temporomandibular joint (TMJ). Magnetic resonance imaging is the gold standard for diagnosis and treatment planning in the disorders of articular disk.[22,23] With the use of MRI, examinations are performed with closed and opened mouth, and mostly static images are taken with an uncomfortable and expensive instrument. Ultrasound (US) examinations overcome these limitations.[24] This non-ionizing imaging method is less expensive, more comfortable for the patient, and can easily be used in a dental setting.[23]

Radiographic examinations are essential in diagnosis and treatment planning in dentistry, but panoramic radiography is less reliable than 3D images, and is not recommended for measurements due to the lack of repeatability.[21,25–27] Cone-beam computed tomography has a wide range of applications for evaluating dental fractures and cracks, measuring the size of periapical lesions, assessing bone density in lesion areas, conducting endodontic surgery, planning implants, and analyzing TMJs and resorption.[28]

High-resolution CBCT has become notably effective for confirming anatomical variations in the mandibular canal. It also makes it possible to precisely locate and view the mental foramen.[29,30]

The occurrence of RMC affects treatment. Knowledge about its prevalence, topography and structure facilitates treatment, especially during all the procedures requiring the use of anesthesia. It is important to provide treatment in the retromolar area with caution to avoid the complications resulting from damage to its contents.[1] Complications may arise from insufficient anesthesia, excessive bleeding, hematomas, and damage to the branches of the inferior alveolar nerve, which may lead to dysfunction of the innervated muscles.[4,11] Blood vessel injuries in the retromolar region during surgery may lead to excessive bleeding in the presence of RMC.[31] Khoury and Hanser observed that 1.44% of their patients had heavy bleeding at the donor site.[32] This bleeding can be locally managed by crushing the bone in the area occupied by the canal or filling the opening with bone wax or bone chips.[32] During the elevation of the mucoperiosteal flap, damage may occur to the neurovascular contents of RMC. This leads to paresthesia of the areas supplied by the retromolar nerve.[4]

Prosthetic restorations (dentures or dental implants) placed distally to the retromolar area may impinge on the contents of RMC. This may cause discomfort and pain or paresthesia, and is especially significant in the elderly due to the alveolar bone resorption.[12] Surgical procedures involving the retromolar region, such as dental implant surgery, impacted third molar extraction and sagittal split ramus osteotomy, can be more complicated.[3] The most common complication after the removal of third molars is inflammation, especially during surgical extraction with root separation.[33] There are reports suggesting that such surgery should be performed using a triangular flap, which reduces the risk of postoperative complications. It is also vital to focus on the bone structure of the mandibular alveolar process, especially in the retromolar area. Retromolar bone dissection should be limited, and bone removal at the base of the coronoid process of the mandible should be avoided.[1]

Due to the presence of the canal, anesthetizing the inferior alveolar nerve may result in insufficient anesthesia.[11] This is called the 'escape pain phenomenon'. This escape pain is due to the nerve contents of RMC.[12] The Gow–Gates technique or the Akinosi method can be used to anesthetize the inferior alveolar nerve when RMC is present.[4,34–37] However, these methods of anesthesia should only be used when traditional local anesthesia has failed.[12]

Conclusions

The present study showed that RMCs were not an infrequent structure. The prevalence of RMCs was 10.5% in the current research group. The most common RMC type in this Polish population was type A. In addition, there was a gender predilection in the studied group, as RMCs were more common in men (F:M = 5.2%:21.2%).

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

Magdalena Piskórz https://orcid.org/0000-0003-4092-1122
Aleksandra Bukiel https://orcid.org/0000-0002-6014-5581
Karolina Kania https://orcid.org/0000-0003-2067-3846
Dorota Kałkowska https://orcid.org/0000-0003-2948-4267
Ingrid Różyło-Kalinowska https://orcid.org/0000-0001-5162-1382

References

1. Komarnitki I, Mańkowska-Pliszka H, Roszkiewicz P, Chloupek A. A morphological study of retromolar foramen and retromolar canal of modern and medieval population. *Folia Morphol*. 2020;79(3):580–587. doi:10.5603/FM.a2019.0124
2. Kim HJ, Kang H, Seo YS, Kim DK, Yu SK. Anatomic evaluation of the retromolar canal by histologic and radiologic analyses. *Arch Oral Biol*. 2017;81:192–197. doi:10.1016/j.archoralbio.2017.05.012
3. Bilecenoglu B, Tuncer N. Clinical and anatomical study of retromolar foramen and canal. *J Oral Maxillofac Surg*. 2006;64(10):1493–1497. doi:10.1016/j.joms.2006.05.043
4. Truong MK, He P, Adeeb N, Oskouian RJ, Tubbs RS, Iwanaga J. Clinical anatomy and significance of the retromolar foramina and their canals: A literature review. *Cureus*. 2017;9(10):e1781. doi:10.7759/cureus.1781
5. Hou Y, Feng G, Lin W, Wang R, Yuan H. Observation of retromolar canals on cone beam computed tomography. *Oral Radiol*. 2020;36(4):365–370. doi:10.1007/s11282-019-00414-0
6. Von Arx T, Hänni A, Sendi P, Buser D, Bornstein MM. Radiographic study of the mandibular retromolar canal: An anatomic structure with clinical importance. *J Endod*. 2011;37(12):1630–1635. doi:10.1016/j.joen.2011.09.007
7. Patil S, Matsuda Y, Nakajima K, Araki K, Okano T. Retromolar canals as observed on cone-beam computed tomography: Their incidence, course, and characteristics. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2013;115(5):692–699. doi:10.1016/j.oooo.2013.02.012
8. D'Apuzzo, F, Minervini G, Grassia V, Rotolo RP, Perillo L, Nucci L. Mandibular coronoid process hypertrophy: Diagnosis and 20-year follow-up with CBCT, MRI and EMG evaluations. Appl Sci. 2021;11(10:4504. doi:10.3390/app11104504
9. Minervini G, Romano A, Petruzzi M, et al. Oral-facial-digital syndrome (OFD): 31-year follow-up management and monitoring. *J Biol Regul Homeost Agents*. 2018;32(2 Suppl 1):127–130. PMID:29460530.
10. Minervini G, Romano A, Petruzzi M, et al. Telescopic overdenture on natural teeth: Prosthetic rehabilitation on (OFD) syndromic patient and a review on available literature. *J Biol Regul Homeost Agents*. 2018;32(2 Suppl 1):131–134. PMID:29460531.
11. Zhou X, Gao X, Zhang J. Bifid mandibular canals: CBCT assessment and macroscopic observation. *Surg Radiol Anat*. 2020;42(9):1073–1079. doi:10.1007/s00276-020-02489-5
12. Ngeow WC, Chai WL. The clinical significance of the retromolar canal and foramen in dentistry. *Clin Anat*. 2021;34(4):512–521. doi:10.1002/ca.23577
13. Ahmed S, Iaturiya R, Ahmad A, Tapasle P, Satpute A, Nagargoje G. Evaluation and incidence of retromolar canal with CBCT in adult population – a retrospective study. MIDSR J Dent Res. 2018;1(1):1–7. https://journal.mitmidsr.edu.in/public/pdf/volume_1_issue_1/evaluation_and_incidence_of_retromolar_canal_with_CBCT_in_adult_population_a_retrospective_study.pdf. Accessed October 11, 2021.
14. Han SS, Hwang YS. Cone beam CT findings of retromolar canal in a Korean population. *Surg Radiol Anat*. 2014;36(9):871–876. doi:10.1007/s00276-014-1262-1
15. Okumuş Ö, Dumlu A. Prevalence of bifid mandibular canal according to gender, type and side. *J Dent Sci*. 2019;14(2):126–133. doi:10.1016/j.jds.2019.03.009
16. Kikuta S, Iwanaga J, Nakamura K, Hino K, Nakamura M, Kusukawa J. The retromolar canals and foramina: Radiographic observation and application to oral surgery. *Surg Radiol Anat*. 2018;40(6):647–652. doi:10.1007/s00276-018-2005-5
17. Jamalpour M, Shokri A, Falah-Koshki S, Zavareian A. Evaluation of retromolar canals with cone beam-computed tomography in an Iranian adult population: A retrospective study. *Int J Clin Dent*. 2016;9(4):233–240.
18. Narayana K, Nayak UA, Ahmed WN, Bhat JG, Devaiah BA. The retromolar foramen and canal in South Indian dry mandibles. *Eur J Anat*. 2002;6(3):141–146.
19. Shirine F, Manikandan L. CBCT in dentistry – an overview. *Eur J Mol Clin Med*. 2020;7(5):1403–1408. https://ejmcm.com/article_4125_c62fd20441647e85a1e42f873a479b65.pdf. Accessed October 11, 2021.
20. Kaufman E, Serman NJ, Wang PD. Bilateral mandibular accessory foramina and canals: A case report and review of the literature. *Dentomaxillofac Radiol*. 2000;29(3):170–175. doi:10.1038/sj/dmfr/4600526

21. Venkatesh E, Elluru SV. Cone beam computed tomography: Basics and applications in dentistry. *J Istamb Univ Fac Dent*. 2017;51(3 Suppl 1):102–121. doi:10.17096/jiufd.00289
22. Minervini G, Nucci L, Lanza A, Femiano F, Contaldo M, Grassia V. Temporomandibular disc displacement with reduction treated with anterior repositioning splint: A 2-year clinical and magnetic resonance imaging (MRI) follow-up. *J Biol Regul Homeost Agents*. 2020;34(1 Suppl 1):151–160. PMID:32064850.
23. Severino M, Caruso S, Rastelli S, et al. Hand-carried ultrasonography instrumentation in the diagnosis of temporomandibular joint dysfunction. *Methods Protoc*. 2021;4(4):81. doi:10.3390/mps4040081
24. Kirkhus E, Gunderson RB, Smith HJ, et al. Temporomandibular joint involvement in childhood arthritis: Comparison of ultrasonography-assessed capsular width and MRI-assessed synovitis. *Dentomaxillofac Radiol*. 2016;45(8):20160195. doi:10.1259/dmfr.20160195
25. Tassoker M, Akin D, Aydin Kabakci AD, Sener S. Comparison of cone-beam computed tomography and panoramic radiography for mandibular morphometry. *Folia Morphol (Warsz)*. 2019;78(4):862–870. doi:10.5603/FM.a2019.0031
26. Sun Z, Smith T, Kortam S, Kim DG, Tee BC, Fields H. Effect of bone thickness on alveolar bone-height measurements from cone-beam computed tomography images. *Am J Orthod Dentofacial Orthop*. 2011;139(2):e117–e127. doi:10.1016/j.ajodo.2010.08.016
27. Lyra Porto OC, de Freitas Silva BS, Silva JA, et al. CBCT assessment of bone thickness in maxillary and mandibular teeth: An anatomic study. *J Appl Oral Sci*. 2020;28:e20190148. doi:10.1590/1678-7757-2019-0148
28. Doğan MS, Callea M, Kusdhany LS, et al. The evaluation of root fracture with cone beam computed tomography (CBCT): An epidemiological study. *J Clin Exp Dent*. 2018;10(1):e41–e48. doi:10.4317/jced.54009
29. Mahmoud Badry MS, El-Badawy FM, Hamed WM. Incidence of retromolar canal in Egyptian population using CBCT: A retrospective study. *Egypt J Radiol Nucl Med*. 2020;51:46. doi:10.1186/s43055-020-00162-w
30. Elkersh NM, Talaab MR, Ahmed WM, Gaweesh YS. Utility of cone beam computed tomogrpahy of the mandible in detection of osteoporosis in postmenopausal women. *Alex Dent J*. 2019;44:46–51. doi:10.21608/adjalexu.2019.57355
31. Fukami K, Shiozaki K, Mishima A, Kuribayashi A, Hamada Y, Kobayashi K. Bifid mandibular canal: Confirmation of limited cone beam CT findings by gross anatomical and histological investigations. *Dentomaxillofac Radiol*. 2012,41(6):460–465. PMID:22116121.
32. Khoury F, Hanser T. Mandibular bone block harvesting from the retromolar region: A 10-year prospective clinical study. *Int J Oral Maxillofac Implants*. 2015;30(3):688–697. doi:10.11607/jomi.4117
33. Kiencało A, Jamka-Kasprzyk M, Panaś M, Wyszyńska-Pawelec G. Analysis of complications after the removal of 339 third molars. *Dent Med Probl*. 2021;58(1):75–80. doi:10.17219/dmp/127028
34. Piskórz M, Hamruk E, Portka K, Różyło-Kalinowska I, Retromolar canal – an essential structure that is often forgotten. *J Stoma*. 2021;74(1):46–49. doi:10.5114/jos.2021.104698
35. Malik S, Sunita, Choudhary A. Clinical and anatomical study of retromolar foramen on adult dry mandible in Uttarakhand region in India. *Int J Cur Res Rev*. 2018;10(16):5–7. doi:10.31782/IJCRR.2018.10162
36. Nikkerdar N, Golshah A, Norouzi M, Falah-Kooshki S. Incidence and anatomical properties of retromolar canal in an Iranian population: A cone-beam computed tomography study. *Int J Dent*. 2020;2020:9178973. doi:10.1155/2020/9178973
37. Zhang YQ, Zhao YN, Liu DG, Meng Y, Ma XC. Bifid variations of the mandibular canal: Cone beam computed tomography evaluation of 1000 Northern Chinese patients. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2018;126(5):e271–e278. doi:10.1016/j.oooo.2018.06.008

Original paper

Additional benefits of titanium platelet-rich fibrin (T-PRF) with a coronally advanced flap (CAF) for recession coverage: A case series

Hirak Shubhra Bhattacharya[1,A,E,F], Shiva Shankar Gummaluri[2,A–F], Avantika Rani[3,B,E], Satyaki Verma[1,B–D], Preeti Bhattacharya[4,E,F], Shiva Manjunath Rayashettypura Gurushanth[5,D–F]

[1] Department of Periodontology and Implantology, Institute of Dental Sciences, Bareilly, India
[2] Department of Periodontology and Implantology, GITAM Dental College and Hospital, Visakhapatnam, India
[3] Department of Periodontology and Implantology, Seema Dental College and Hospital, Rishikesh, India
[4] Department of Orthodontics and Dentofacial Orthopedics, Institute of Dental Sciences, Bareilly, India
[5] Department of Periodontology and Implantology, Rajendra Institute of Medical Sciences (RIMS), Ranchi, India

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):279–285

Address for correspondence
Shiva Shankar Gummaluri
E-mail: sivashankar.gummaluri@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on May 17, 2021
Reviewed on August 19, 2021
Accepted on September 3, 2021

Published online on June 29, 2023

Cite as
Bhattacharya HS, Gummaluri SS, Rani A, Verma S, Bhattacharya P, Rayashettypura Gurushanth SM. Additional benefits of titanium platelet-rich fibrin (T-PRF) with a coronally advanced flap (CAF) for recession coverage: A case series. *Dent Med Probl.* 2023;60(2):279–285. doi:10.17219/dmp/141919

DOI
10.17219/dmp/141919

Abstract

Background. Platelet concentrates (PCs) are a boon in the field of dentistry. Various generations of PCs have been tried and utilized in different treatment methods, such as intrabony defect therapy, root coverage procedures, oral surgical procedures, and palatal wound healing. Titanium-prepared platelet-rich fibrin (T-PRF) is a third-generation PC that is prepared in medical-grade titanium tubes and achieves good healing in the field of periodontics.

Objectives. Not many studies have been performed utilizing T-PRF in the treatment of gingival recession (GR). The present case series study aimed to evaluate the efficacy of T-PRF in the treatment of Cairo's Type 1 GR defects.

Material and methods. A total of 20 patients with 34 Cairo's Type 1 GR defects were recruited. The surgical sites were treated using the trapezoidal coronally advanced flap (CAF) technique and T-PRF as a biomaterial underneath the flap. The plaque index (PI) and the gingival index (GI), recession depth (RD) and recession width (RW), as well as the width of keratinized tissue (WKT), were measured at baseline and 6 months postoperatively. The obtained values were subjected to statistical analysis. The values were presented as mean (*M*) and standard deviation (*SD*), the paired t test was performed to measure all the parameters, and a p-value <0.05 was considered to be statistically significant.

Results. The changes observed 6 months after the use of T-PRF were non-significant for PI ($p = 0.053$) and significant for GI ($p = 0.016$) as compared to the baseline. Significant reductions ($p < 0.001$) were noted for RD and RW, as well as a significant increase in WKT and a mean root coverage (MRC) of 91%.

Conclusions. Titanium-prepared platelet-rich fibrin can be used as a biomaterial for the treatment of GR defects, as it eliminates the possible silica contamination, as in the case of leukocyte-platelet-rich fibrin (L-PRF), and the need for a second surgical site, as with subepithelial connective tissue graft (SCTG). Moreover, the use of T-PRF results in a thicker membrane formation, and titanium tubes can be reused after proper sterilization.

Keywords: titanium, chronic periodontitis, gingival recession, platelet-rich fibrin

Introduction

The recession of gingiva can be described as the migration of gingiva apically below the cementoenamel junction (CEJ), causing the exposure of the root surface. It is always a tough task to treat and manage gingival recession (GR).[1] The main reason for the occurrence of GR may be faulty toothbrushing techniques causing trauma, poor oral hygiene causing plaque accumulation, gingival inflammation, and the anatomical variations of a buccally or labially placed tooth, where thinner buccal or labial bone causes GR.[2] Apart from these, the thickness of gingiva also plays a role; in thinner and scalloped tissue, higher chances of GR are reported.[3]

Various treatment modalities, such as free gingival graft (FGG),[4] coronally advanced flap (CAF), semilunar coronally positioned flap (SCPF),[5] subepithelial connective tissue graft (SCTG)[6] or a combination of CAF + SCTG, resorbable and non-resorbable membranes,[7] like amnion/chorion membranes and collagen membranes, and platelet concentrates (PCs),[8] like platelet-rich plasma (PRP), leukocyte-platelet-rich fibrin (L-PRF), advanced platelet-rich fibrin (A-PRF), and acellular dermal matrix (ADM) allograft[9] had been tried, and showed good long-term results regarding complete recession coverage and gain in the width of keratinized tissue (WKT). Yet later on, researchers used more conservative surgical techniques, like envelop flap, vestibular incision subperiosteal tunnel access (VISTA), modified VISTA, and combination approaches.[10] The modified coronally advanced tunnel (MCAT)[11] and laterally closed tunnel (LCT)[12] techniques are the recent advancements in soft tissue surgery for the treatment of multiple or single isolated deep GR sites, with good results being reported regarding recession coverage and improved clinical parameters.

The usage of biomaterials has improved the outcomes of recession coverage procedures. This statement is supported by a recent systematic review done by Chamberone et al.[13] The authors concluded that any biomaterial + CAF improved treatment outcomes in terms of complete root coverage, being comparable to CAF + SCTG, which is considered to be the gold standard in recession treatment.[13] Moreover, CAF alone would result in decreased postoperative root coverage percentage over time.[14] The abovementioned commercially available materials are very costly, and in this context, PCs are a boon to dentists, as they are readily available, easy to prepare, and they eliminate the second surgical site in SCTG procurement. Initially, PRP alone and in combination with SCTG or CAF were tried, but due to the drawbacks of PRP, such as the addition of an anticoagulant and hypersensitivity, L-PRF in combination with CAF were used, yielding increased WKT and full mean root coverage (MRC).[15] Later on, due to silica contamination through the glass test tubes and silica-coated Vacutainer® tubes used for the preparation of this PC, researchers again started searching for a better biomaterial.[16,17] The search has resulted in the development of a third-generation PC called titanium-prepared platelet-rich fibrin (T-PRF).[18]

Titanium-prepared platelet-rich fibrin was introduced by Tunalı et al. in 2013,[18] following a similar pattern of preparation of L-PRF given by Choukroun et al. in 2001.[19] Instead of glass tubes, medical-grade titanium tubes were used. Moreover, titanium has better hemocompatibility, activates platelets similarly to silica, and the tubes can be reused after proper sterilization and are unbreakable. Histological studies done by Chatterjee et al.[20] and Bhattacharya et al.[21] concluded that T-PRF showed a thicker membrane meshwork and better entrapment of cells, which might increase the regenerative capacity. Apart from this, T-PRF also has a greater percentage of platelets, monocytes and lymphocytes, and equal amounts of progenitor cells when compared with L-PRF.[21] Furthermore, the PC contains growth factors, like transforming growth factor beta (TGF-β), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF), which accelerate the healing process and allow to achieve the required treatment outcomes.[22] A recent study done by Uzun et al. compared CAF + connective tissue graft (CTG) and CAF + T-PRF, and achieved good results in terms of recession coverage and gain in WKT in both cases.[23]

Since not many studies have been performed with the use of T-PRF, the present study aimed to evaluate the efficacy of T-PRF as a biomaterial along with conventional CAF in the treatment of Cairo's Type 1 GR defects.

Material and methods

Sample size calculation

The sample size was calculated using the G*Power software, v. 3.1 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). At a power of 80%, an effect size of 0.25 and an α-value of 5%, a sample of 17 was sufficient to conduct the current research. For a better outcome, 34 recession sites were treated in the present study.

Study design and patient enrollment

The present study is a prospective, single-centered, single-blinded case series. Patients were recruited from the outpatient clinic at the Department of Periodontology and Implantology of the Institute of Dental Sciences, Bareilly, India. Initially, 25 patients were examined for a study population, out of which 5 were eliminated, as 2 were not willing to participate and 3 did not meet the criteria for inclusion. Thus, a total of 20 systemically healthy patients (14 males and 6 females) with a mean age of 30.3 ±10.11 years and 34 Cairo's Type 1 GR sites[24] were recruited,

treated and followed up for 6 months in the present study. The research was approved by the Institutional Ethics Committee (IEC/IDS/152/2021), and informed consent was obtained from all patients prior to the commencement of the study. The study was performed from September 2020 to November 2020. Amidst the coronavirus disease 2019 (COVID-19) pandemic, all precautions were taken, with the proper sterilization of instruments and the operation theater area, and further self-protection of the operator and the patient. Pros and cons regarding the procedure were explained to the patients. The study was conducted in accordance with the 1975 Declaration of Helsinki, modified in 2008.

Inclusion and exclusion criteria

The inclusion criteria comprised patients with an age range of 18–45 years, having Cairo Type 1 GR defects[24] in maxillary teeth (anterior and premolars) without mobility, who were systemically healthy, did not undergo any periodontal treatment within the last 6 months, and were not using any medications that would hamper healing. Furthermore, the eligible patients were those who visited the periodontist mainly because they were seeking treatment for their GR. Patients having pockets deeper than 3 mm, any malposition of the teeth that needed to be treated, any systemic illness, smokers, alcoholics, pregnant and lactating females, patients with a platelet count (PLT) < 2,000,000/mm^3, patients with GR Type 2 or 3 of Cairo's classification, patients with any mobility in the teeth of concern, and patients with the absence of the adjacent teeth, where CAF could rest, were excluded from the study.

Clinical parameters

The plaque index (PI),[25] the gingival index (GI)[26], recession depth (RD), recession width (RW), and WKT were assessed at baseline and 6 months postoperatively, using the CP15 University of North Carolina probe (UNC-15) (Equinox Instruments Ltd, Lincoln, UK). While measuring, CEJ is taken as the guide, and if CEJ is not visible, the CEJ of the adjacent tooth is taken into account. Prior to making the final measurements of the main study, the examiner evaluated the readings of 5 random patients who were not included in the study within a gap of 72 h. If the variation between the values was ±1, then the final outcome values had an accuracy of 90%, and only the examiner was allowed to take measurements in the main study.[27] The mean root coverage percentage (MRC%) was calculated at the 6-month follow-up based on root coverage, using the following formula (Equation 1):

$$\text{MRC\%} = \frac{\text{preoperative RD} - \text{postoperative RD}}{\text{preoperative RD}} \times 100\% \quad (1)$$

where:
MRC% – mean root coverage percentage; and
RD – recession depth.

Pain perception was recorded using a visual analog scale (VAS; a scoring range of 1 to 10) 24 h after surgery and on the 7th postoperative day.[28] These measurements were taken via the telephone by calling the patient. Due to the COVID-19 pandemic, recall visits were limited, and patients could be recalled only in emergency cases.

Pre-surgical procedure

Patients who were willing to undergo surgery were considered, and the initial phase I therapy, which included scaling and root planing, was performed. Then, oral hygiene instructions (OHI) were provided, soft brushes were advocated and the modified Stillman's brushing technique was demonstrated to the patients. Upon re-evaluation after 6–8 weeks,[29] if the patient maintained the lower PI and GI scores (≤1), they were qualified for the surgical procedure. Before surgery, routine blood investigations and COVID-19 reverse-transcription polymerase chain reaction (RT-PCR) tests were performed to rule out abnormalities.

Preparation of T-PRF clots

Just 20 min before surgery, 10 mL of blood was drawn from the antecubital vein and directly transferred into sterile medical-grade titanium tubes (Supra Alloys, Camarillo, USA). The blood was subjected to centrifugation in a centrifuge machine (Remi R-8C; India MART, New Delhi, India) at 3,500 rotations per minute for 15 min.[18] Three layers were formed within the test tubes, with the top layer being the supernatant serum, the lower layer being the red blood cell suspension and the T-PRF clots in the middle layer. The clots were carefully retrieved by using sterile tweezers and compressed into a thin membrane by placing them in between sterile gauze pieces so that the excessive serum could be eliminated. The obtained membranes were placed at the recession sites.

Surgical procedure

The extraoral and intraoral antiseptic procedures were performed using 0.5 w/v% povidone-iodine and 0.2% chlorhexidine gluconate (CHX; Rexidine®; Indoco Remedies Ltd., Mumbai, India), respectively. Before performing the surgical procedure, the baseline measurements were made by a blinded experienced periodontal surgeon (SSG), and the surgical procedure was performed by a different periodontist (SV). After the achievement of profound anesthesia (local infiltrations were administered at the surgical site by using 2% lidocaine hydrochloride with adrenaline 1:80,000), RD was measured where measurements were necessary for the horizontal

incisions (the distal and mesial line angles of the recession of the treated tooth/teeth) and the interdental papilla was spared. These horizontal incisions were then connected with sulcular incisions. The vertical releasing incisions (VRIs) were performed on both sides of the treated site and connected with the horizontal incisions without involving normal tooth/teeth. The incisions were made using a No. 15 blade. Thus, a trapezoidal flap was reflected and VRIs were helpful in the advancement of the flap. A full-thickness mucoperiosteal flap was reflected by using a periosteal elevator up to the mucogingival junction (MGJ), and a partial-thickness flap was reflected beyond MGJ into the alveolar mucosa. The flap was carefully repositioned coronally and checked for muscle tension or strain. If the flap was advanced without any tension, the root surface was carefully planed to remove any debris and calculus remnants so that a smooth surface was obtained. Later on, the abovementioned freshly prepared T-PRF membranes were placed onto the denuded surface. The flap was repositioned coronally and stabilized with a sling suture, and VRIs were approximated with simple interrupted sutures. A periodontal pack (Coe-Pack™; GC Asia Dental, Hyderabad, India) was placed to protect the treated area from any abnormalities of the tongue or muscle tissue and food particles. The patients were recalled after 14 days for suture removal. The type of suture material used in the present study was non-resorbable 4-0 silk sutures. The type of surgical technique employed in the present study was described by Zuchelli et al.[30]

Post-surgical procedure

After the surgery, the patient was re-educated on strict oral hygiene principles, and medications were prescribed – amoxicillin 500 mg thrice daily for 5 days to prevent postoperative bacteremia, and diclofenac + paracetamol twice daily on the 1st day and whenever necessary from the 2nd day onward to the 5th day. A CHX mouthwash (Rexidine) was prescribed at 24 h of surgery twice daily for a period of 14 days. The patient was called via the telephone after 1 day (24 h) to assess the VAS score for postoperative pain. The VAS pain assessment was repeated on the 7th day, and after 14 days, the patient was recalled for suture removal. After that, irrigation with betadine and saline was performed, and the patient was again educated on oral hygiene principles and told to refrain from brushing the surgical area for an additional 2 weeks. During this time, a wet cotton pad dipped in a CHX solution was used to clean the surgical site from soft tissue to the crown side so that debris did not accumulate. The patient was given instructions for normal regular brushing with the use of the modified Bass technique 1 month after surgery. The patient remained in follow-up and was recalled after 6 months to study the condition and gather the postoperative measurements. Midterm recall visits were reduced because of the COVID-19 pandemic.

The procedures described above, as well as the follow-up, are illustrated in Fig. 1.

Fig. 1. Baseline (preoperative) recession (A), the incision design (B), the images of titanium tubes (C,D), the titanium-prepared platelet-rich fibrin (T-PRF) membrane (E), the reflection of the flap and the placement of the T-PRF membrane (F), suturing at the surgical site (G); the placement of Coe-Pak (H), 15 days post-op (I), and 6 months post-op (J)

Statistical analysis

All the gathered data was transferred to a Microsoft Excel spreadsheet, and statistical analysis was carried out with the use of IBM SPSS Statistics for Windows, v. 22.0 (IBM Corp., Armonk, USA). All parameter values were presented as mean (*M*) and standard deviation (*SD*). The paired *t* test was performed to find significant differences in the PI, GI, RD, RW, and WKT values 6 months postoperatively with regard to baseline. The mean root coverage was expressed as percentage (%).

Results

The mean age, gender distribution, the type of recession, and the number of teeth treated are depicted as demographic data (Table 1). Regarding PI, there was no significant difference between the baseline values and those recorded 6 months postoperatively (p = 0.053),

Table 1. Demographic data of the study group

Demographic characteristics		Data
Age [years] *M* ±*SD*		30.3 ±10.11
Gender *n* (%)	M	14 (70.0)
	F	6 (30.0)
Cairo's Type 1 GR		34
Number of maxillary anterior/premolar teeth		19/15

M – mean; *SD* – standard deviation; *n* – number; GR – gingival recession; M – male; F – female.

though a reduction was noticed. However, there was a statistically significant reduction in the GI values from baseline to 6 months postoperatively (p = 0.016). There were highly significant reductions in RD and RW, whereas significant gain was reported in WKT when the values were compared at baseline and 6 months postoperatively (p < 0.001). There was also a significant reduction in the VAS scores when the values were compared at 24 h of surgery on the 7th postoperative day (p < 0.001). The MRC% value was 91% (Table 2).

Table 2. Comparison of various parameters at baseline and 6 months post-op in the study group

Variable	Baseline *M ±SD*	6 months post-op *M ±SD*	MD	p-value
PI	0.75 ±0.28	0.64 ±0.16	0.11	0.053
GI	0.69 ±0.21	0.59 ±0.14	0.10	0.016*
RD [mm]	3.05 ±1.10	0.25 ±0.55	2.80	<0.001*
RW [mm]	3.55 ±0.60	0.55 ±1.19	3.00	<0.001*
WKT [mm]	3.00 ±1.21	4.25 ±0.72	1.25	<0.001*
VAS score	5.20 ±0.77	1.85 ±0.67	3.35	<0.001*
MRC%		91%		

PI – plaque index; GI – gingival index; RD – recession depth; RW – recession width; WKT – width of keratinized tissue; MRC% – mean root coverage percentage; *MD* – mean difference; * statistically significant.

Discussion

The present study describes the use of T-PRF as a biomaterial in the treatment of GR. There was uneventful healing in the treated sites, without any complications, and no patient was lost to the follow-up. The biomaterial used formed a thicker membrane, which helped in better entrapment of cells and growth factors, and resulted in in better healing.[31] Titanium-prepared platelet-rich fibrin was previously used in the treatment of intrabony defects by Chatterjee et al.[32], Arabaci et al.[31], Mitra et al.[33], and Gummaluri et al.[34] All researchers achieved a reduction in pocket depth (PD), gain in the clinical attachment level (CAL), and improved defect fill and resolution.[31–34] The current study data was compared with the available literature on the usage of T-PRF as a biomaterial in the treatment of GR, which is scarce.

In the present study, there were lower PI scores (non-significantly) and GI scores (significantly) at a 6-month follow-up as compared to baseline. The finding regarding PI is in contrast to the results obtained by Koyuncuoğlu et al.[11] and Uzun et al.,[23] whereas for GI, they reported comparable observations. In our study, the lower values of PI could not reach statistical significance. Sutures were used to maintain good tensile strength without tension, and the application of postoperative CHX mouthwash and good OHI to maintain proper oral hygiene caused lower plaque accumulation, which in turn reduced gingival inflammation. Good compliance and attitude toward treatment helped achieve successful results.[35]

In the present study, significant reductions were reported regarding RD and RW, which is in harmony with recent studies conducted by Koyuncuoğlu et al.[11] and Uzun et al.[23] There was an increase in WKT of 1.25 mm in the present study, which is almost equal to the results obtained in the abovementioned studies, where the increases amounted to 1.97 mm[11] and 2 mm[23] at 6 months, and 2.2 mm at 3 years.[11,23] These variations might be due to smaller sample sizes and the type of surgical technique employed in their studies. The present study utilized the trapezoidal technique, whereas the researchers mentioned above used the tunneling procedure. The increased WKT and decreased RD and RW might be due to the thicker membrane that was formed during centrifugation, leading to a thicker fibrin mesh with a higher and constant release of growth factors, as greater cellularity leads to improved parameters.[21] Moreover, the conventional CAF utilized in the present study yielded similar outcomes as the tunneling techniques (the modified CAF and MCAT techniques[11]) which have been used in the recent era.

There was a 91% MRC recorded in the present study, which is in accordance with recent studies. Koyuncuoğlu et al.[11] and Uzun et al.[23] reported scores of 93.10% and 93.29%, respectively, and concluded that T-PRF produced similar results to SCTG. The present study MRC% is in harmony with their SCTG group, which is considered to be the gold standard. In their recent systematic reviews, Miron et al.[36] and Moraschini and Dos Santos Porto Barboza[37] considered L-PRF for the treatment of GR and concluded that although it was helpful in achieving a considerable percentage of relative root coverage (RRC), it provided no additional benefit in terms of increasing the width of keratinized mucosa (WKM); it was recommended that SCTG be used if the baseline WKM value was reduced. The present study used T-PRF in CAF and showed similar results to SCTG. Thus, T-PRF could be a better alternative to L-PRF in the treatment of GR defects. Apart from this, T-PRF also eliminated the second surgical site and postoperative pain. Though the present study had a shorter 6-month follow-up, it was sufficient to assess the outcome of the treatment, as stated by Jepsen et al.[38]. This might be due to the relocation of MGJ to its original position being completed in 6 months. Also, T-PRF was evaluated in palatal wound healing by Ustaoğlu et al., who achieved good results.[39] Therefore, T-PRF can be an alternative histoconduction material to SCTG in the treatment of GR.

In regard to esthetics, patient satisfaction and fewer postoperative complications, like the absence of dentinal hypersensitivity and swelling, as well as achieving good root coverage, are very important. The present study met most of these criteria, and patients were totally satisfied regarding the outcomes of the treatment.[28]

Limitations

Despite the good outcomes reported, there were some inevitable limitations to the study. Only maxillary-arch teeth were considered, the healing index was not considered, histological sectioning was not performed, a small sample size was used, the gingival thickness measurements were not taken, and the conventional technique of CAF was used, where VRIs might hamper the blood supply and cause scarring. The higher cost of titanium tubes also reduces their usage in clinical practice. Long-term split-mouth randomized controlled trials (RCTs) with SCTG as a control group compared with the conservative tunneling surgical techniques might help identify the efficacy of T-PRF in the treatment of GR.

Conclusions

Within the limitations of the present study, T-PRF can be used as a biomaterial in the treatment of GR, as it eliminates silica contamination (L-PRF with silica-coated or glass test tubes) and the need for a second surgical site (SCTG procurement), reducing postoperative pain. Titanium tubes can be reused after proper sterilization Moreover, the thicker fibrin structure resulted in better root coverage and a good WKT. So, in the near future, T-PRF may be regarded as a better choice among PC preparations in periodontal treatment.

Ethics approval and consent to participate

The research was approved by the Institutional Ethics Committee (IEC/IDS/152/2021), and informed consent was obtained from all patients prior to the commencement of the study. The study was conducted in accordance with the 1975 Declaration of Helsinki, modified in 2008.

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

Hirak Shubhra Bhattacharya
https://orcid.org/0000-0001-8518-0597
Shiva Shankar Gummaluri https://orcid.org/0000-0003-3892-7322
Avantika Rani https://orcid.org/0000-0002-2583-391X
Satyaki Verma https://orcid.org/0000-0002-9400-4648
Preeti Bhattacharya https://orcid.org/0000-0001-9537-4192
Shiva Manjunath Rayashettypura Gurushanth
https://orcid.org/0000-0001-7634-0288

References

1. Jati AS, Furquim LZ, Consolaro A. Gingival recession: Its causes and types, and the importance of orthodontic treatment. *Dental Press J Orthod*. 2016;21(3):18–29. doi:10.1590/2177-6709.21.3.018-029
2. Shah N, Mathur VP, Jain V, Logani A. Association between traditional oral hygiene methods with tooth wear, gingival bleeding, and recession: A descriptive cross-sectional study. *Indian J Dent Res*. 2018;29(2):150–154. doi:10.4103/ijdr.IJDR_651_16
3. Abraham S, Deepak KT, Ambili R, Preeja C, Archana V. Gingival biotype and its clinical significance – a review. *Saudi J Dent Res*. 2014;5(1):3–7. doi:10.1016/j.ksujds.2013.06.003
4. Miller PD Jr. Root coverage using the free soft tissue autograft following citric acid application. II. Treatment of the carious root. *Int J Periodontics Restorative Dent*. 1983;3(5):38–51. PMID:6581145.
5. Tarnow DP. Semilunar coronally repositioned flap. *J Clin Periodontol*. 1986;13(3):182–185. doi:10.1111/j.1600-051x.1986.tb01456.x
6. Langer B, Langer L. Subepithelial connective tissue graft technique for root coverage. *J Periodontol*. 1985;56(12):715–720. doi:10.1902/jop.1985.56.12.715
7. Amarante ES, Leknes KN, Skavland J, Lie T. Coronally positioned flap procedures with or without a bioabsorbable membrane in the treatment of human gingival recession. *J Periodontol*. 2000;71(6):989–998. doi:10.1902/jop.2000.71.6.989
8. Tunalı M, Özdemir H, Arabacı T, Gürbüzer B, Pikdöken ML, Firatli E. Clinical evaluation of autologous platelet-rich fibrin in the treatment of multiple adjacent gingival recession defects: A 12-month study. *Int J Periodontics Restorative Dent*. 2015;35(1):105–114. doi:10.11607/prd.1826
9. Barootchi S, Tavelli L, Di Gianfilippo R, et al. Acellular dermal matrix for root coverage procedures: 9-year assessment of treated isolated gingival recessions and their adjacent untreated sites. *J Periodontol*. 2021;92(2):254–262. doi:10.1002/JPER.20-0310
10. Fernández-Jiménez A, Estefanía-Fresco R, García-De-La-Fuente AM, Marichalar-Mendia X, Aguirre-Zorzano LA. Description of the modified vestibular incision subperiosteal tunnel access (m-VISTA) technique in the treatment of multiple Miller class III gingival recessions: A case series. *BMC Oral Health*. 2021;21(1):142. doi:10.1186/s12903-021-01511-5
11. Koyuncuoğlu CZ, Ercan E, Uzun B, Tunali M, Firatli E. Management of deep gingival recessions by modified coronally advanced tunnel technique with titanium platelet rich fibrin membrane or connective tissue graft: 36 months follow-up clinical study. *Clin Exp Health Sci*. 2020;10(3):297–303. doi:10.33808/marusbed.767457
12. Sculean A, Allen EP. The laterally closed tunnel for the treatment of deep isolated mandibular recessions: Surgical technique and a report of 24 cases. *Int J Periodontics Restorative Dent*. 2018;38(4):479–487. doi:10.11607/prd.3680
13. Chambrone L, Salinas Ortega MA, Sukekava F, et al. Root coverage procedures for treating single and multiple recession-type defects: An updated Cochrane systematic review. *J Periodontol*. 2019;90(12):1399–1422. doi:10.1002/JPER.19-0079
14. Chambrone L, Salinas Ortega MA, Sukekava F, et al. Root coverage procedures for treating localised and multiple recession-type defects. *Cochrane Database Syst Rev*. 2018;10(10): CD007161. doi:10.1002/14651858.CD007161.pub3
15. Mancini L, Tarallo F, Quinzi V, Fratini A, Mummolo S, Marchetti E. Platelet-rich fibrin in single and multiple coronally advanced flap for Type 1 recession: An updated systematic review and meta-analysis. *Medicina (Kaunas)*. 2021;57(2):144. doi:10.3390/medicina57020144
16. Tsujino T, Takahashi A, Yamaguchi S, et al. Evidence for contamination of silica microparticles in advanced platelet-rich fibrin matrices prepared using silica-coated plastic tubes. *Biomedicines*. 2019;7(2):45. doi:10.3390/biomedicines7020045
17. O'Connell SM. Safety issues associated with platelet-rich fibrin method. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007;103(5):587–593. doi:10.1016/j.tripleo.2007.03.017
18. Tunalı M, Özdemir H, Küçükodacı Z, Akman S, Fıratlı E. In vivo evaluation of titanium-prepared platelet-rich fibrin (T-PRF): A new platelet concentrate. *Br J Oral Maxillofac Surg*. 2013;51(5):438–443. doi:10.1016/j.bjoms.2012.08.003
19. Choukroun J, Adda F, Schoeffler C, Vervelle A. Une opportunité en paro-implantologie: Le PRF. *Implantodontie*. 2001;42(55):e55–e62.
20. Chatterjee A, Debnath K, Ali MM, Babu C, Gowda PL. Comparative histologic evaluation of titanium platelet-rich fibrin and platelet-rich fibrin in hypertensive and smoker participants: A cell cytology study. *J Indian Soc Periodontol*. 2017;21(3):195–200. doi:10.4103/jisp.jisp_137_17

21. Bhattacharya HS, Gummaluri SS, Astekar M, Gummaluri RK. Novel method of determining the periodontal regenerative capacity of T-PRF and L-PRF: An immunohistochemical study. *Dent Med Probl*. 2020;57(2):137–144. doi:10.17219/dmp/117721
22. Tunalı M, Özdemir H, Küçükodacı Z, et al. A novel platelet concentrate: Titanium-prepared platelet-rich fibrin. *Biomed Res Int*. 2014;2014:209548. doi:10.1155/2014/209548
23. Uzun BC, Ercan E, Tunalı M. Effectiveness and predictability of titanium-prepared platelet-rich fibrin for the management of multiple gingival recessions. *Clin Oral Investig*. 2018;22(3):1345–1354. doi:10.1007/s00784-017-2211-2
24. Cairo F, Nieri M, Cincinelli S, Mervelt J, Pagliaro U. The interproximal clinical attachment level to classify gingival recessions and predict root coverage outcomes: An explorative and reliability study. *J Clin Periodontol*. 2011;38(7):661–666. doi:10.1111/j.1600-051X.2011.01732.x
25. Löe H, Silness J. Periodontal disease in pregnancy I. Prevalence and severity. *Acta Odontol Scand*. 1963;21:533–551. doi:10.3109/00016356309011240
26. Löe H. The gingival index, the plaque index and the retention index systems. *J Periodontol*. 1967;38(6 Suppl):610–616. doi:10.1902/jop.1967.38.6.610
27. Akcan SK, Ünsal B. Gingival recession treatment with concentrated growth factor membrane: A comparative clinical trial. *J Appl Oral Sci*. 2020;28:e20190236. doi:10.1590/1678-7757-2019-0236
28. Agarwal MC, Kumar G, Shiva Manjunath RG, Sai Karthikeyan SS, Gummaluri SS. Pinhole surgical technique – a novel minimally invasive approach for treatment of multiple gingival recession defects: A case series. *Contemp Clin Dent*. 2020;11(1):97–100. doi:10.4103/ccd.ccd_449_19
29. Segelnick SL, Weinberg MA. Reevaluation of initial therapy. When is the appropriate time? *N Y State Dent J*. 2007;73(2):46–49. PMID:17472186.
30. Zucchelli G, Mele M, Mazzotti C, Marzadori M, Montebugnoli L, De Sanctis M. Coronally advanced flap with and without vertical releasing incisions for the treatment of multiple gingival recessions: A comparative controlled randomized clinical trial. *J Periodontol*. 2009;80(7):1083–1094. doi:10.1902/jop.2009.090041
31. Arabaci T, Albayrak M. Titanium-prepared platelet-rich fibrin provides advantages on periodontal healing: A randomized split-mouth clinical study. *J Periodontol*. 2018;89(3):255–264. doi:10.1002/JPER.17-0294
32. Chatterjee A, Pradeep AR, Garg V, Yajamanya S, Ali MM, Priya VS. Treatment of periodontal intrabony defects using autologous platelet-rich fibrin and titanium platelet-rich fibrin: A randomized, clinical, comparative study. *J Investig Clin Dent*. 2017;8(3). doi:10.1111/jicd.12231
33. Mitra DK, Potdar PN, Prithyani SS, Rodrigues SV, Shetty GP, Talati MA. Comparative study using autologous platelet-rich fibrin and titanium prepared platelet-rich fibrin in the treatment of infrabony defects: An in vitro and in vivo study. *J Indian Soc Periodontol*. 2019;23(6):554–561. doi:10.4103/jisp.jisp_562_18
34. Gummaluri SS, Bhattacharya HS, Astekar M, Cheruvu S. Evaluation of titanium-prepared platelet-rich fibrin and leucocyte platelet-rich fibrin in the treatment of intra-bony defects: A randomized clinical trial. *J Dent Res Dent Clin Dent Prospects*. 2020;14(2):83–91. doi:10.34172/joddd.2020.020
35. Solderer A, Kaufmann M, Hofer D, Wiedemeier D, Attin T, Schmidlin PR. Efficacy of chlorhexidine rinses after periodontal or implant surgery: A systematic review. *Clin Oral Investig*. 2019;23(1):21–32. doi:10.1007/s00784-018-2761-y
36. Miron RJ, Moraschini V, Del Fabbro M, et al. Use of platelet-rich fibrin for the treatment of gingival recessions: A systematic review and meta-analysis. *Clin Oral Investig*. 2020;24(8):2543–2557. doi:10.1007/s00784-020-03400-7
37. Moraschini V, Dos Santos Porto Barboza E. Use of platelet-rich fibrin membrane in the treatment of gingival recession: A systematic review and meta-analysis. *J Periodontol*. 2016;87(3):281–290. doi:10.1902/jop.2015.150420
38. Jepsen K, Stefanini M, Sanz M, Zucchelli G, Jepsen S. Long-term stability of root coverage by coronally advanced flap procedures. *J Periodontol*. 2017;88(7):626–633. doi:10.1902/jop.2017.160767
39. Ustaoğlu G, Ercan E, Tunali M. The role of titanium-prepared platelet-rich fibrin in palatal mucosal wound healing and histoconduction. *Acta Odontol Scand*. 2016;74(7):558–564. doi:10.1080/00016357.2016.1219045

Original paper

Orthodontic treatment need, the types of brackets and the oral health-related quality of life

Judith Patricia Barrera-Chaparro[A,C–F], Sonia Patricia Plaza-Ruíz[A,C–F], Karen Lorena Parra[B,C,E,F], Magda Quintero[B,C,E,F], María Del Pilar Velasco[B,C,E,F], María Carolina Molinares[B,C,E,F], Catalina Álvarez[B,C,E,F]

Fundación Universitaria CIEO (UniCIEO), Bogota, Colombia

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):287–294

Address for correspondence
Judith Patricia Barrera-Chaparro
E-mail: jp.barrera@unicieo.edu.co

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
The authors would like to thank Dr. Rodrigo Rivera (the Coordinator of the Department of Research) for his advice.

Received on March 23, 2022
Reviewed on May 21, 2022
Accepted on June 24, 2022

Published online on June 30, 2023

Abstract

Background. Malocclusion can affect the oral health-related quality of life (OHRQoL). The influence of the orthodontic treatment need (OTN) and the type of brackets on OHRQOL is not clear.

Objectives. The aim of the present study was to determine the relationships between OTN and the bracket type and OHRQoL during the first 6 months of orthodontic treatment (OT) in adult patients.

Material and methods. This cohort study was conducted at the Department of Orthodontics of a private university. A total of 216 patients aged ≥18 years participated in the study (106 patients with conventional brackets and 110 with self-ligating brackets). The OHRQoL was evaluated using the 14-item Oral Health Impact Profile (OHIP-14) at 5 time points – before OT (T0), and at 24/48 h (T1), 1 month (T2), 3 months (T3), and 6 months (T4) after the installation of the orthodontic appliance. The OTN was evaluated with the dental aesthetic index (DAI) by 2 previously calibrated operators. For the statistical analysis, the χ^2 test and the Mann–Whitney *U* test were used. Additionally, Poisson regression models were performed.

Results. The evidence of an association between OHRQoL and OTN was found only at T3 ($p = 0.0095$). No association was found between OHRQoL and the bracket type. However, in the regression models, OHRQoL was statistically significantly worse at T3 in the group with a greater OTN (*IRR* (incidence rate ratio) = 1.34; 95% *CI* (confidence interval): 1.21;1.48) and at T4 in the self-ligation group (*IRR* = 1.23; 95% *CI*: 1.12;1.36).

Conclusions. The OHRQoL was affected in the same way at the beginning of OT, regardless of OTN and the bracket type used. However, a worse OHRQoL was observed at 3 months in subjects with greater OTN and at 6 months in patients with self-ligating brackets.

Keywords: malocclusion, orthodontic appliances, quality of life, orthodontics, orthodontic brackets

Cite as
Barrera-Chaparro JP, Plaza-Ruíz SP, Parra KL, et al. Orthodontic treatment need, the types of brackets and the oral health-related quality of life. *Dent Med Probl.* 2023;60(2):287–294. doi:10.17219/dmp/151577

DOI
10.17219/dmp/151577

Introduction

Malocclusion can lead to the discrimination of individuals based on their facial appearance and to low self-esteem, which can affect the oral health-related quality of life (OHRQoL).[1–3] However, orthodontic treatment (OT) with labial fixed appliances can cause a temporary decline in OHRQoL.[4–6] Adult patients undergoing treatment with fixed orthodontic appliances are most likely to experience increased levels of pain for 1–3 days following the placement of the appliance and subsequent visits for adjustments.[7] Chen et al. assessed changes in OHRQoL during fixed orthodontic appliance therapy in Chinese patients, and found that the compromised condition in terms of overall OHRQoL was most severe during the first week after bracket placement.[8] Zhang et al. reported significant improvement in OHRQoL after 6 months of OT.[9]

There are some differences between orthodontic treatment in children and adults due to different basic oral health conditions, therapeutic compliance and oral hygiene.[10–12] Furthermore, adult patients may have compromised occlusion or temporomandibular disorders, which is why more cautious OT should be set up.[13,14] Additionally, there are difficulties in bracket bonding in adult patients due to the presence of prosthetic elements or other conditions in which adhesion could be compromised, like syndromes with impaired tooth mineralization.[15–17]

Self-ligating brackets have been advertised to have many reputed advantages with regard to diminishing discomfort and pain levels during initial orthodontic therapy.[18] However, there is no evidence suggesting that self-ligating brackets are more comfortable and lead to less severe pain than conventional brackets.[19] Few studies have compared the impact of conventional and self-ligating brackets on OHRQoL during OT, and no statistically significant differences were found.[20–23] However, Zhou et al. observed that patients with self-ligating brackets experienced less severe pain and discomfort than those with conventional brackets.[20] Likewise, Othman et al. demonstrated that passive self-ligating, active self-ligating and conventional brackets differed in terms of pain and discomfort at the time of placement.[22]

Slade and Spencer proposed the Oral Health Impact Profile (OHIP) as an instrument to measure OHRQoL,[24] which is widely used in dentistry and has been validated in numerous languages.[25–31] The long form of this tool has 49 items (OHIP-49) and evaluates 7 dimensions (i.e., functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability, and handicap).

Although untreated malocclusion has been shown to have a negative impact on OHRQoL, malocclusion severity seems to dictate the need for OT.[2,23] In subjects with mild malocclusion, OT is elective, and minor occlusal traits may not have as large of a negative impact on OHRQoL as severe malocclusion. The influence of both orthodontic treatment need (OTN) and the bracket type on OHRQOL is not clear. The present study aimed to evaluate the relationships between OTN and the bracket type and OHRQoL during the first 6 months of OT in adult patients.

Material and methods

This prospective cohort study included a sample of 216 adult patients – 110 patients in the self-ligation group (mean age: 31.75 ±11.63 years; 60 females and 50 males) and 106 patients in the conventional group (mean age: 29.26 ±9.62 years; 61 females and 45 males) (Table 1) who were selected by consecutive sampling and were treated at the Department of Orthodontics of the UniCIEO University, Bogota, Colombia, from December 27, 2016, to May 29, 2019. The study protocol was approved by the ethics committee of the University (No. 007/46 as of November 16, 2016). All patients gave informed written consent for participating in the study. The research was conducted in full accordance with the World Medical Association (WMA) Declaration of Helsinki.

The sample size was calculated based on the data from a pilot study that included 60 patients (35 with self-ligating and 25 with conventional brackets). Epidat 4.2 (Xullo 2016, Consellería de Sanidade, Xunta de Galicia, España; Organización Panamericana da Saúde (OPS-OMS), Universidade CES, Colombia) was used for the calculation, considering a confidence interval (*CI*) of 95% and a power of 90%. At least 91 subjects were required for each group to detect a mean difference in the 14-item Oral Health Impact Profile (OHIP-14) total score of 2 points (±3.76 and ±4.45 for the self-ligating and conventional brackets, respectively). The inclusion criteria were subjects who were 18 years old or older, and did not have extractions, surgical interventions or mini-screw insertion at the beginning of the treatment or within 1 month before the survey was administered. Patients with craniofacial anomalies, caries, systemic diseases, uncontrolled periodontal disease, OT, cognitive disorders, and those who did not understand the Spanish language were excluded.

The outcome measure was OHRQoL, which was measured with a previously validated Spanish version of OHIP-14.[25] To ensure the transcultural compatibility of OHIP-14, an expert committee (4 orthodontists and 4 orthodontic patients) assessed its face validity (whether the instrument appears to be measuring the variables it claims to measure) and content validity (whether the scale components cover all the attributes to be measured). The OHIP-14 scale was a Likert-type scale with the following scores: 4 – very often; 3 – fairly often; 2 – occasionally; 1 – hardly ever; and 0 – never. The total score was calculated as the sum of the item scores, generating scores from 0 to 56, with a higher score indicating a more negative impact and a lower OHRQoL.[32]

Table 1. Distribution of the variables in the study groups at baseline

Variable		Bracket type		*p*-value	OTN		*p*-value
		conventional (*n* = 106)	self-ligating (*n* = 110)		lesser	greater	
Age *M* ±*SD*		29.26 ±9.62	31.75 ±11.63	0.1045‡	29.27 ±9.97	33.22 ±11.86	0.0293*‡
Gender *n* (%)	M	45 (47.37)	50 (52.63)	0.6570†	63 (66.32)	32 (33.68)	0.6270†
	F	61 (50.41)	60 (49.59)		84 (69.42)	37 (30.58)	
DAI (total score) *M* ±*SD*		28.39 ±8.62	28.18 ±9.37	0.8308‡			
OTN *n* (%)	lesser	31 (44.93)	38 (55.07)	0.4040†			
	greater	75 (51.02)	72 (48.98)				

M – mean; *SD* – standard deviation; *n* – number; M – male; F – female; DAI – dental aesthetic index; OTN – orthodontic treatment need; * statistically significant ($p < 0.05$); † χ^2 test; ‡ Mann–Whitney *U* test.

The scale was administered by 3 examiners (KLP, MQ and MdPV) at 5 time points: before OT (T0), and at 24/48 h (T1), 1 month (T2), 3 months (T3), and 6 months (T4) after the installation of the orthodontic appliance. To ensure the reliability of the information, the examiners were trained to administer the surveys. The first survey was conducted through personal interviews, and the remaining surveys were completed through telephone interviews by the same researchers.

The predictor variables were OTN and the bracket type. The self-ligating bracket group had a 0.022 × 0.027-inch slot (50 patients with SmartClip™ (3M Unitek, Monrovia, USA) and 60 patients with Carriere® SLX (Ortho Organizers, Carlsbad, USA)), and the conventional bracket group had a 0.022 × 0.028-inch slot and the MBT prescription (Gemini brackets; 3M Unitek). Although the patients were treated by different operators, the operators followed a pre-established clinical protocol with the same treatment philosophy. Additionally, data on the age and gender of the patients were collected from the surveys.

The OTN was evaluated with the dental aesthetic index (DAI) and measured in dental casts by 2 examiners (MQ and MdPV) who were previously trained by an expert operator on the index in accordance with the World Health Organization (WHO) manual methods.[33] The DAI measures 10 occlusal traits (the number of visible missing teeth, incisor crowding, the interdental incisor space, the width of the midline diastema, maxillary tooth irregularity, mandibular tooth irregularity, incisor overjet, mandibular overjet, anterior open bite, and the buccal segment relationship), each of which is multiplied by a predetermined statistical weight, and a constant of 13 points is added to obtain the total score of DAI.[34] Jenny and Cons established the cut-off points for the following categories to assess malocclusion severity and OTN: no abnormalities or minor malocclusion (13–25); definite malocclusion (26–30); severe malocclusion (31–35); and very severe or handicapping malocclusion (>36).[35] In the present study, this variable was dichotomized based on the cut-off score to demarcate the need for orthodontic services that have been previously defined.[36] Subjects with DAI higher than 30 were considered to have a greater OTN, and subjects with DAI between 13 and 30 were considered to have a lesser OTN.

The reliability of the DAI measurements was determined in 20 cast models randomly selected from the sample and measured on 2 occasions with a 1-week interval. Intra- and interobserver agreement was evaluated with the Bland–Altman plot. Additionally, the method error was estimated with the paired *t* test (the systematic error) and Dahlberg's formula (the random error).[37]

Statistical analysis

All analyses were performed with Stata software, v.14 (StataCorp, College Station, USA). To evaluate the associations between categorical variables, the χ^2 test was applied. Nonparametric tests were used because of the non-normal distribution of the data. The Mann–Whitney *U* test was used to evaluate the association between OTN, the bracket type and OHRQoL across the time intervals. Additionally, 5 multiple logistic Poisson regression analyses with the variables OTN and the bracket type included in the model were performed to evaluate their associations with OHRQoL. The significance level was established at $p < 0.05$.

The random errors were within acceptable limits, and there were no statistically significant systematic errors ($p > 0.1$). The Bland–Altman plots indicated high intraobserver agreement, with an average error between –0.38 and 0.48 points in the total DAI (95% *CI*: –0.48–0.70).

Results

The response rate to the questionnaires was 100%. At baseline, there were no statistically significant differences in age, gender, the total DAI, and OTN between the bracket type groups ($p > 0.05$). Likewise, there were no significant associations between OTN and gender (Table 1).

The associations between OTN and the bracket type and OHRQoL (OHIP-14 total score) at different time points are reported in Table 2. The only statistically significant association was found between OTN and OHRQoL at T3 (p = 0.0095), showing a worse OHRQoL in the group with greater OTN (8.80) than in the group with lesser OTN (6.54) (Fig. 1A,1B).

The relative changes in OHRQoL at different time points were compared according to the bracket type (Table 3), but there were no statistically significant differences for any of the time intervals. The mean relative changes with 95% *CI*s in OHRQoL across the time intervals are described in Table 4. Greater differences were observed in the T1–T0 (24–48 h after bracket placement) and T2–T0 (1 month after bracket placement) time intervals in the OHIP-14 total scores, and especially for the dimensions of physical pain and physical disability.

The Poisson regression models (one for each time point), which included the OHIP-14 total score as the outcome variable and OTN and the bracket type as the predictor variables, showed that 3 months after appliance placement (T3), having a greater OTN increased the incidence rate ratio (*IRR*) of having a worse OHRQoL by 1.34 times (95% *CI*: 1.21;1.48). Additionally, patients with self-ligating brackets at 6 months after appliance placement (T4) were more likely to have a worse OHRQoL than those who used conventional brackets (*IRR* = 1.23; 95% *CI*: 1.12;1.36) (Table 5).

Table 3. Relative changes in the oral health-related quality of life (OHRQoL) at different time points according to the bracket type

OHIP-14 total score across time intervals	Bracket type		*p*-value
	conventional	self-ligating	
T1–T0	5.85 ±8.10	6.37 ±8.70	0.4921
T2–T0	2.93 ±7.98	2.41 ±7.83	0.3598
T3–T0	0.43 ±7.22	0.59 ±8.10	0.9365
T4–T0	0.10 ±8.33	1.22 ±9.64	0.5546
T2–T1	–2.92 ±7.05	–3.95 ±6.12	0.0889
T3–T2	–2.50 ±5.30	–1.83 ±5.24	0.3596
T4–T3	–0.33 ±6.85	0.64 ±8.17	0.6775

Data presented as *M* ±*SD*. Time points: T0 – before orthodontic treatment (OT); T1 – at 24/48 h after the installation of the orthodontic appliance; T2 – at 1 month after the installation of the orthodontic appliance; T3 – at 3 months after the installation of the orthodontic appliance; and T4 – at 6 months after the installation of the orthodontic appliance. Mann–Whitney *U* test.

Table 2. Associations between the orthodontic treatment need (OTN) and the bracket type and the oral health-related quality of life (OHRQoL) (OHIP-14 total score) across the time intervals

OHIP-14 total score at different time points	OTN		*p*-value	Bracket type		*p*-value
	lesser	greater		conventional	self-ligating	
T0	6.87 ±7.12	6.50 ±5.70	0.9076	6.53 ±7.01	6.96 ±6.39	0.3754
T1	12.70 ±7.98	13.23 ±7.70	0.4073	12.39 ±8.11	13.34 ±7.65	0.2089
T2	9.17 ±6.54	9.94 ±6.21	0.2882	9.46 ±6.32	9.38 ±6.58	0.8924
T3	6.54 ±4.95	8.80 ±6.40	0.0095**	6.96 ±5.55	7.54 ±5.55	0.3244
T4	7.40 ±6.89	7.45 ±6.79	0.8809	6.62 ±6.21	8.69 ±7.34	0.1564

Data presented as *M* ±*SD*. Time points: T0 – before orthodontic treatment (OT); T1 – at 24/48 h after the installation of the orthodontic appliance; T2 – at 1 month after the installation of the orthodontic appliance; T3 – at 3 months after the installation of the orthodontic appliance; and T4 – at 6 months after the installation of the orthodontic appliance. ** statistically significant (p < 0.01); Mann–Whitney *U* test.

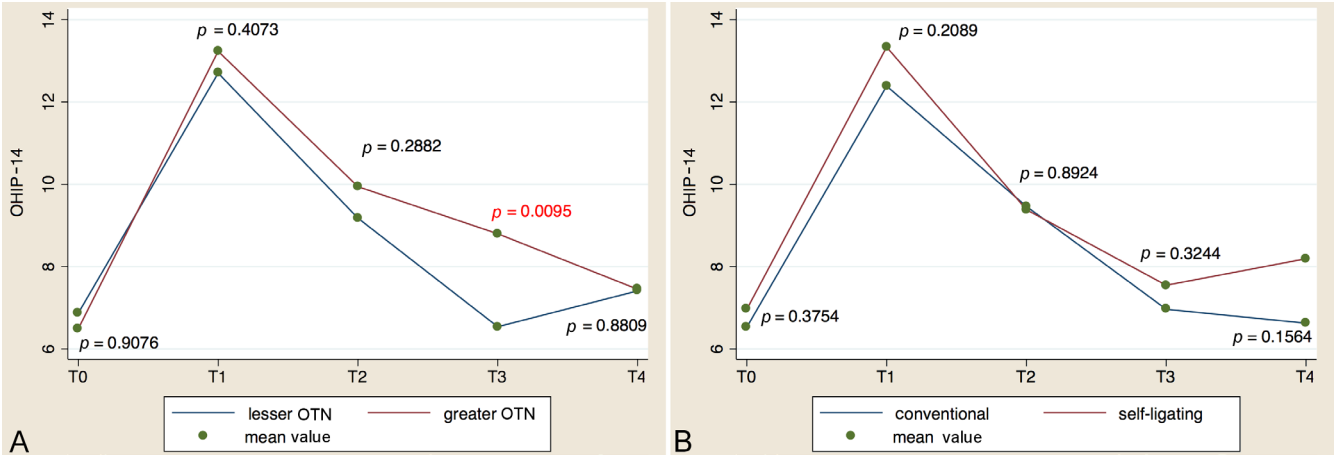

Fig. 1. Oral health-related quality of life (OHRQoL) across the time intervals according to the orthodontic treatment need (OTN) (A) and according to the bracket type (B)

Time points: T0 – before orthodontic treatment (OT); T1 – at 24/48 h after the installation of the orthodontic appliance; T2 – at 1 month after the installation of the orthodontic appliance; T3 – at 3 months after the installation of the orthodontic appliance; and T4 – at 6 months after the installation of the orthodontic appliance. OHIP-14 – 14-item Oral Health Impact Profile.

Table 4. Mean relative changes with 95% confidence interval (*CIs*) in the oral health-related quality of life (OHRQoL) across the time intervals

Variable	T1–T0	T2–T0	T3–T0	T4–T0	T2–T1	T3–T2	T4–T3
OHIP-14 total score	6.12 (4.99;7.24)	2.67 (1.61;3.72)	0.51 (–0.52;1.54)	0.67 (–0.54;1.88)	–3.45 (–4.33;–2.56)	–2.16 (–2.86;–1.45)	0.16 (–0.85;1.17)
Functional limitation	0.93 (0.72;1.14)	0.65 (0.45;0.85)	0.50 (0.31;0.68)	0.53 (0.31;0.75)	–0.28 (–0.48;–0.08)	–0.15 (–0.33;–0.03)	–0.03 (–0.18;0.25)
Physical pain	2.94 (2.59;3.29)	2.05 (1.74;2.35)	1.28 (0.98;1.58)	1.27 (0.94;1.60)	–0.89 (–1.18;–0.60)	–0.77 (–1.01;–0.52)	–0.10 (–0.26;0.02)
Psychological discomfort	–0.49 (–0.80;–0.18)	–0.99 (–1.32;–0.66)	–1.41 (–1.70;–1.11)	–1.27 (–1.58;–0.96)	–0.50 (–0.73;–0.27)	–0.41 (–0.61;–0.22)	0.14 (–0.11;0.38)
Physical disability	2.61 (2.29;2.92)	1.87 (1.57;2.17)	1.28 (0.99;1.56)	0.93 (0.64;1.22)	–0.74 (–1.02;–0.44)	–0.60 (–0.83;–0.36)	–0.34 (–0.60;–0.08)
Psychological disability	–0.09 (–0.33;0.15)	–0.59 (–0.81;–0.38)	–0.91 (–1.12;–070)	–0.76 (–0.99;–0.53)	–0.50 (–0.70;–0.31)	–0.31 (–0.47;–0.16)	0.15 (–0.03;0.32)
Social disability	0.22 (0.31;0.40)	–0.09 (–0.24;0.04)	–0.02 (–0.17;0.12)	0.04 (–0.14;0.23)	–0.31 (–0.46;–0.16)	0.07 (–0.05;0.19)	0.06 (–0.10;0.23)
Handicap	0.00 (–0.16;0.15)	–0.18 (–0.31;–0.06)	–0.18 (–0.31;–0.06)	–0.02 (–0.18;0.13)	–0.18 (–0.30;–0.06)	0.00 (–0.08;0.08)	0.16 (0.26;0.30)

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

Table 5. Multivariate adjusted models using the Poisson regression with the incidence rate ratio (*IRR*) for the association of the oral health-related quality of life (OHRQoL) at different time points with the orthodontic treatment need (OTN) and the bracket type

Variable			*IRR* (95% *CI*)	*p*-value
OHIP-14 T0	OTN	lesser	1	0.286
		greater	0.94 (0.84;1.05)	
	bracket type	conventional	1	0.197
		self-ligating	1.07 (0.96;1.19)	
OHIP-14 T1	OTN	lesser	1	0.366
		greater	1.04 (0.96;1.12)	
	bracket type	conventional	1	0.059
		self-ligating	1.07 (0.10;1.16)	
OHIP-14 T2	OTN	lesser	1	0.085
		greater	1.08 (0.99;1.19)	
	bracket type	conventional	1	0.772
		self-ligating	0.99 (0.90;1.08)	
OHIP-14 T3	OTN	lesser	1	<0.0001****
		greater	1.34 (1.21;1.48)	
	bracket type	conventional	1	0.206
		self-ligating	1.07 (0.10;1.18)	
OHIP-14 T4	OTN	lesser	1	0.891
		greater	0.99 (0.89;1.10)	
	bracket type	conventional	1	<0.0001****
		self-ligating	1.23 (1.12;1.36)	

**** statistically significant ($p < 0.0001$).

Discussion

In the present study, we found no significant differences between conventional and self-ligating brackets in terms of their impact on a patient's OHRQoL. However, the mean OHIP-14 overall scores were higher in the self-ligating bracket group than in the conventional bracket group at T1–T0, T3–T0, T4–T0, T3–T2, and T4–T3. Similar results were found by Lai et al.[21] In contrast, Zhou et al. observed that self-ligating brackets were associated with less severe pain and discomfort at any time point as compared to conventional brackets, but the differences between groups were not significant.[20] Likewise, Othman et al. found no significant differences between the types of brackets; however, the passive self-ligating and active self-ligating bracket groups showed more immediate and delayed effects in the bonding phase, respectively, and the conventional bracket group was affected in both assessments.[22] Many authors have studied the association between OT and the quality of life during different stages of OT.[5,8,9,20,38–40] In orthodontics, the first stage of treatment is alignment and leveling, which usually takes 4–6 months, depending on the amount of crowding, and continues until the stainless-steel archwires are in place. Such archwires are more rigid and help to complete the torque expression. Changing archwires may affect the quality of life through causing more discomfort in the patient. Also, after this stage, the teeth and the smile normally look better, which may influence the OHIP-14 score due to the OHIP-14 scale measuring not only pain and discomfort, but also psychological and social disability.

In the present study, when evaluating the association between OHRQoL and OTN, we found significant declines in OHRQoL at T3 in patients who had a greater OTN ($p = 0.0095$). This result may be explained by the idea that most of the necessary alignment and leveling may have resolved 3 months after bracket installation in individuals with mild to moderate malocclusion. In contrast, it may take longer to resolve crowding or other occlusal traits in individuals with severe malocclusion, and the use of more biomechanics tools or adjustments may lead to a worse OHRQoL. Zheng et al. observed

that changes in OHRQoL followed different patterns among patients with different types of malocclusion.[41] The OHRQoL of Class I malocclusion patients could be significantly improved just after alignment and leveling with OT. Class III patients showed benefits at all stages and Class II patients showed apparent improvement during the space closure stage.[41]

With regard to the relative changes across the time intervals in our study, for almost all the time comparisons with the baseline (T1–T0, T2–T0, T3–T0, and T4–T0), the functional limitation, physical pain and physical disability dimensions showed worse scores at 24–48 h after bracket placement (T1); then, the scores gradually improved and nearly reached the baseline values at 6 months after bracket placement (T4). At T4, the OHIP-14 overall and dimension scores were very low, which suggests that at 6 months after bracket placement, the level of OHRQoL of patients tends to be restored to that before OT. Similar results have been reported by many authors,[9,20–22] and as in this study, the functional limitation, physical pain and physical disability dimensions showed worse scores at 24–48 h after bracket placement (T1–T0). Nevertheless, the social disability and handicap dimensions did not show significant differences over time. Similar results were found in other studies,[4,8,20–22,41] which concluded that the main problems during OT were difficulty in chewing foods, tooth pain and sensitivity, the misunderstanding of some words, appearance being affected, and smiling. Social skills (the social disability dimension), financial loss or the inability to function (the handicap dimension) were not affected during OT.

However, one of the main differences between our research and other studies[20–22] is that we included the study variable OTN as a predictor variable in addition to the bracket type, and studied the interaction between these variables and OHRQoL. According to the regression model, in which the bracket type was included, at 3 months after appliance placement (T3), the group with a greater OTN showed an increase in the *IRR* of having a higher OHIP-14 score (*IRR* = 1.34; 95% *CI*: 1.21;1.48). This finding suggests that at 3 months after bracket placement, subjects with more severe malocclusion had a greater chance of perceiving a poorer OHRQoL than those with less severe malocclusion. Furthermore, at 6 months after appliance placement (T4), a deterioration in the OHIP-14 overall score is expected in patients with self-ligating brackets as compared to those with conventional brackets (*IRR* = 1.23; 95% *CI*: 1.12;1.36). This observation may be explained by the standard arch sequence at this time, as rectangular stainless-steel wires are being inserted, which can cause more discomfort and/or pain in patients with self-ligation brackets. Mansor et al. found the highest prevalence and severity of the immediate impact on the OHIP overall score in the conventional bracket group at 6 weeks after bracket placement when rectangular wires were inserted, showing a poorer OHRQoL at that time.[42]

Limitations and clinical implications

One limitation of this study was that the appliances used in the self-ligating group comprised those of different brands, including active and passive self-ligation brackets. However, many authors have not found differences between these two appliances in terms of clinical[42] or perceived comfort.[22] Also, the short time interval between the observations for the measurement error in DAI could explain the high intraobserver agreement. As to the clinical implications of our findings, patients should be informed that self-ligating brackets are not different from conventional brackets in terms of the resulting OHRQoL, that they are going to suffer a decline in OHRQoL in the first months of OT, which differs according to patients' OTN at 3 months and according to the bracket type at 6 months after appliance installation, and that after 6 months they will feel the same as they did before OT.

Conclusions

No differences were found between the types of brackets during the first 6 months of OT. According to the multinomial regression, OTN and the bracket type led to important changes during OT at 3 months and 6 months, respectively, worsening the patients' OHRQoL at each of the corresponding time intervals. Significant changes in the OHRQoL of the subjects occurred over time after bracket placement – OHRQoL decreased considerably at 24–48 h, and subsequently improved to the baseline value at 6 months after orthodontic appliance insertion.

Ethics approval and consent to participate

The study protocol was approved by the ethics committee of the University (No. 007/46 as of November 16, 2016). All patients gave informed written consent for participating in the study. The research was conducted in full accordance with the World Medical Association (WMA) Declaration of Helsinki.

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

Judith Patricia Barrera-Chaparro https://orcid.org/0000-0002-6308-7241
Sonia Patricia Plaza-Ruíz https://orcid.org/0000-0002-4577-3096
Karen Lorena Parra https://orcid.org/0000-0002-4707-4790
Magda Quintero https://orcid.org/0000-0002-7228-1222
María Del Pilar Velasco https://orcid.org/0000-0001-8904-2722
María Carolina Molinares https://orcid.org/0000-0002-1098-183X
Catalina Álvarez https://orcid.org/0000-0001-7397-1041

References

1. Healey DL, Gauld RD, Thomson WM. Treatment-associated changes in malocclusion and oral health–related quality of life: A 4-year cohort study. *Am J Orthod Dentofacial Orthop*. 2016;150(5):811–817. doi:10.1016/j.ajodo.2016.04.019
2. Clijmans M, Lemiere J, Fieuws S, Willems G. Impact of self-esteem and personality traits on the association between orthodontic treatment need and oral health-related quality of life in adults seeking orthodontic treatment. *Eur J Orthod*. 2015;37(6):643–650. doi:10.1093/ejo/cju092
3. Hassan AH, El-Sayed Amin H. Association of orthodontic treatment needs and oral health-related quality of life in young adults. *Am J Orthod Dentofacial Orthop*. 2010;137(1):42–47. doi:10.1016/j.ajodo.2008.02.024
4. Yassir YA, McIntyre GT, Bearn DR. The impact of labial fixed appliance orthodontic treatment on patient expectation, experience, and satisfaction: An overview of systematic reviews. *Eur J Orthod*. 2020;42(3):223–230. doi:10.1093/ejo/cjz043
5. Johal A, Fleming PS, Al Jawad FA. A prospective longitudinal controlled assessment of pain experience and oral health-related quality of life in adolescents undergoing fixed appliance treatment. *Orthod Craniofac Res*. 2014;17(3):178–186. doi:10.1111/ocr.12044
6. Liu Z, McGrath C, Hägg U. The impact of malocclusion/orthodontic treatment need on the quality of life a systematic review. *Angle Orthod*. 2009;79(3):585–591. doi:10.2319/042108-224.1
7. Johal A, Ashari AB, Alamiri N, et al. Pain experience in adults undergoing treatment: A longitudinal evaluation. *Angle Orthod*. 2018;88(3):292–298. doi:10.2319/082317-570.1
8. Chen M, Wang DW, Wu LP. Fixed orthodontic appliance therapy and its impact on oral health-related quality of life in Chinese patients. *Angle Orthod*. 2010;80(1):49–53. doi:10.2319/010509-9.1
9. Zhang M, McGrath C, Hägg U. Changes in oral health-related quality of life during fixed orthodontic appliance therapy. *Am J Orthod Dentofacial Orthop*. 2008;133(1):25–29. doi:10.1016/j.ajodo.2007.01.024
10. Contaldo M, Della Vella F, Raimondo E, et al. Early Childhood Oral Health Impact Scale (ECOHIS): Literature review and Italian validation. *Int J Dent Hyg*. 2020;18(4):396–402. doi:10.1111/idh.12451
11. Di Stasio D, Romano A, Paparella RS, et al. How social media meet patients' questions: YouTube™ review for mouth sores in children. *J Biol Regul Homeost Agents*. 2018;32(2 Suppl 1):117–121. PMID:29460528.
12. Di Stasio D, Romano AN, Paparella RS, et al. How social media meet patients' questions: YouTube™ review for children oral thrush. *J Biol Regul Homeost Agents*. 2018;32(2 Suppl 1):101–106. PMID:29460525.
13. Minervini G, Russo D, Herford AS, et al. Teledentistry in the management of patients with dental and temporomandibular disorders. *Biomed Res Int*. 2022;2022:7091153. doi:10.1155/2022/7091153
14. Moccia S, Nucci L, Spagnuolo C, D'Apuzzo F, Piancino MG, Minervini G. Polyphenols as potential agents in the management of temporomandibular disorders. *Appl Sci*. 2020;10(15):5305. doi:10.3390/app10155305
15. Minervini G, Romano A, Petruzzi M, et al. Telescopic overdenture on natural teeth: Prosthetic rehabilitation on (OFD) syndromic patient and a review on available literature. *J Biol Regul Homeost Agents*. 2018;32(2 Suppl 1):131–134. PMID:29460531.
16. Antonelli A, Bennardo F, Brancaccio Y, et al. Can bone compaction improve primary implant stability? An in vitro comparative study with osseodensification technique. *Appl Sci*. 2020;10(23):8623. doi:10.3390/app10238623
17. Minervini G, Romano A, Petruzzi M, et al. Oral-facial-digital syndrome (OFD): 31-year follow-up management and monitoring. *J Biol Regul Homeost Agents*. 2018;32(2 Suppl 1):127–130. PMID:29460530.
18. Scott P, Sherriff M, Dibiase AT, Cobourne MT. Perception of discomfort during initial orthodontic tooth alignment using a self-ligating or conventional bracket system: A randomized clinical trial. *Eur J Orthod*. 2008;30(3):227–332. doi:10.1093/ejo/cjm131
19. Čelar A, Schedlberger M, Dörfler P, Bertl MH. Systematic review on self-ligating vs. conventional brackets: Initial pain, number of visits, treatment time. *J Orofac Orthop*. 2013;74(1):40–51. doi:10.1007/s00056-012-0116-x
20. Zhou Y, Zheng M, Lin J, Wang Y, Ni ZY. Self-ligating brackets and their impact on oral health-related quality of life in Chinese adolescence patients: A longitudinal prospective study. *ScientificWorldJournal*. 2014;2014:352031. doi:10.1155/2014/352031
21. Lai TT, Chiou JY, Lai TC, Chen T, Chen MH. Oral health-related quality of life in orthodontic patients during initial therapy with conventional brackets or self-ligating brackets. *J Dent Sci*. 2017;12(2):161–172. doi:10.1016/j.jds.2016.12.003
22. Othman SA, Mansor N, Saub R. Randomized controlled clinical trial of oral health-related quality of life in patients wearing conventional and self-ligating brackets. *Korean J Orthod*. 2014;44(4):168–176. doi:10.4041/kjod.2014.44.4.168
23. George R, Samson RS, Soe HH, et al. Oral health-related quality of life and the index of orthodontic treatment need to evaluate the association of patients' self-perceived need and normative need toward orthodontic treatment. *J Int Oral Health*. 2018;10(3):115–120. doi:10.4103/jioh.jioh_64_18
24. Slade GD, Spencer AJ. Development and evaluation of the Oral Health Impact Profile. *Community Dent Health*. 1994;11(1):3–11. PMID:8193981.
25. Castrejón-Pérez R, Borges-Yáñez SA. Derivation of the short form of the Oral Health Impact Profile in Spanish (OHIP-EE-14). *Gerodontology*. 2012;29(2):155–158. doi:10.1111/j.1741-2358.2012.00613.x
26. Saub R, Locker D, Allison P. Derivation and validation of the short version of the Malaysian Oral Health Impact Profile. *Community Dent Oral Epidemiol*. 2005;33(5):378–383. doi:10.1111/j.1600-0528.2005.00242.x
27. Corridore D, Campus G, Guerra F, Ripari F, Sale S, Ottolenghi L. Validation of the Italian version of the Oral Health Impact Profile-14 (IOHIP-14). *Ann Stomatol (Roma)*. 2014;4(3–4):239–243. PMID:24611088. PMCID:PMC3935349.
28. Roumani T, Oulis CJ, Papagiannopoulou V, Yfantopoulos J. Validation of a Greek version of the Oral Health Impact Profile (OHIP-14) in adolescents. *Eur Arch Paediatr Dent*. 2010;11(5):247–252. doi:10.1007/BF03262756
29. Montero-Martín J, Bravo-Pérez M, Albaladejo-Martínez A, Hernández-Martín LA, Rosel-Gallardo EM. Validation the Oral Health Impact Profile (OHIP-14sp) for adults in Spain. *Med Oral Patol Oral Cir Bucal*. 2009;14(1):E44–E50. PMID:19114956.
30. León S, Bravo-Cavicchioli D, Correa-Beltrán G, Giacaman RA. Validation of the Spanish version of the Oral Health Impact Profile (OHIP-14Sp) in elderly Chileans. *BMC Oral Health*. 2014;14:95. doi:10.1186/1472-6831-14-95
31. Barrera-Chaparro JP, Plaza-Ruíz SP, Camacho-Usaquén T, Pasuy-Caicedo JA, Villamizar-Rivera AK. Modified short version of the oral health impact profile for patients undergoing orthodontic treatment. *Braz J Oral Sci*. 2021;20:e211717. doi:10.20396/bjos.v20i00.8661717
32. Slade GD. Derivation and validation of a short-form oral health impact profile. *Community Dent Oral Epidemiol*. 1997;25(4):284–290. doi:10.1111/j.1600-0528.1997.tb00941.x
33. World Health Organization (WHO). Oral health surveys: Basic methods. 4th ed. 1997. https://apps.who.int/iris/handle/10665/41905. Accessed March 1, 2022.
34. Cons NC, Jenny J, Kohout FJ. *DAI – The Dental Aesthetic Index*. Iowa City, IA: College of Dentistry, University of Iowa; 1986.
35. Jenny J, Cons NC. Establishing malocclusion severity levels on the Dental Aesthetic Index (DAI) scale. *Aust Dent J*. 1996;41(1):43–46. doi:10.1111/j.1834-7819.1996.tb05654.x

36. Danyluk K, Lavelle C, Hassard T. Potential application of the dental aesthetic index to prioritize the orthodontic service needs in a publicly funded dental. *Am J Orthod Dentofacial Orthop*. 1999;116(3):279–286. doi:10.1016/s0889-5406(99)70239-8
37. Houston WJ. The analysis of errors in orthodontic measurements. *Am J Orthod*. 1983;83(5):382–390. doi:10.1016/0002-9416(83)90322-6
38. Zheng M, Liu R, Ni Z, Yu Z. Efficiency, effectiveness and treatment stability of clear aligners: A systematic review and meta-analysis. *Orthod Craniofac Res*. 2017;20(3):127–133. doi:10.1111/ocr.12177
39. Liu Z, McGrath C, Hägg U. Changes in oral health-related quality of life during fixed orthodontic appliance therapy: An 18-month prospective longitudinal study. *Am J Orthod Dentofacial Orthop*. 2011;139(2):214–219. doi:10.1016/j.ajodo.2009.08.029
40. Johal A, Alyaqoobi I, Patel R, Cox S. The impact of orthodontic treatment on quality of life and self-esteem in adult patients. *Eur J Orthod*. 2015;37(3):233–237. doi:10.1093/ejo/cju047
41. Zheng DH, Wang XX, Su YR, et al. Assessing changes in quality of life using the Oral Health Impact Profile (OHIP) in patients with different classifications of malocclusion during comprehensive orthodontic treatment. *BMC Oral Health*. 2015;15(1):148. doi:10.1186/s12903-015-0130-7
42. Mansor N, Saub R, Othman SA. Changes in the oral health-related quality of life 24 h following insertion of fixed orthodontic appliances. *J Orthod Sci*. 2012;1(4):98–102. doi:10.4103/2278-0203.105880

Original paper

Tribological, microhardness and color stability properties of a heat-cured acrylic resin denture base after reinforcement with different types of nanofiller particles

Shurooq Falih Altaie[A–F]

Department of Prosthetic Dental Techniques, College of Health and Medical Techniques, Middle Technical University, Baghdad, Iraq

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):295–302

Address for correspondence
Shurooq Falih Altaie
E-mail: shurooqfalih94@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on January 24, 2021
Reviewed on May 13, 2021
Accepted on May 18, 2021

Published online on June 1, 2023

Abstract

Background. Polymethyl methacrylate (PMMA) is not an ideal material in all aspects, as it has poor mechanical and antimicrobial properties. The enhancement of the mechanical and physical features of PMMA-based material is highly required.

Objectives. The present study aimed to estimate the effect of adding different types of nanoparticle (NP) materials on the mechanical and physical features of heat-cured acrylic resin denture base material.

Material and methods. A total of 120 samples were divided into 4 groups: the control group; the 5 wt% ZrO_2 NPs group; the 5 wt% TiO_2 NPs group; and the 5 wt% Ag NPs group. Each one was subdivided into 3 groups according to the test performed: the microhardness test; the abrasive wear test; and the color stability test. Then, the results of these tests were evaluated.

Results. The maximum mean value for the microhardness test was observed in the group treated with the addition of 5 wt% Ag NPs, followed by the 5 wt% ZrO_2 NPs group, and finally the 5 wt% TiO_2 NPs group. The lowest mean microhardness value was recorded for the control group. The maximum mean value for the abrasive wear test was attributed to the control group and the lowest mean value was related to the 5 wt% Ag NPs group. The maximum color change was noted in the 5 wt% Ag NPs group, followed by the 5 wt% ZrO_2 NPs and 5 wt% TiO_2 NPs groups. The lowest mean value for the color change was found in the control group.

Conclusions. There was an increase in hardness and wear resistance in the group treated by adding 5 wt% Ag NPs, and the control group had the best color stability, followed by the 5 wt% TiO_2 NPs group. However, a detrimental effect on color stability was observed when 5 wt% Ag NPs was added.

Keywords: nanoparticles, hardness, wear, ZrO_2, reinforcement of an acrylic denture base

Cite as
Altaie SF. Tribological, microhardness and color stability properties of a heat-cured acrylic resin denture base after reinforcement with different types of nanofiller particles. *Dent Med Probl.* 2023;60(2):295–302. doi:10.17219/dmp/137611

DOI
10.17219/dmp/137611

Introduction

Polymethyl methacrylate (PMMA)-based acrylic has been characterized as the most highly favored denture base material.[1] It was introduced in 1937 by W. Wright[2] because of its desirable properties, such as durability, low toxicity, satisfactory esthetics, good stability in oral conditions, low weight, and a low cost.[3] Yet, PMMA is not an ideal material in all aspects, as it has poor surface mechanical properties and limited antimicrobial activity.[4] The enhancement of the physical as well as mechanical qualities of PMMA-based material is highly required.[5] A recently developed procedure can improve the mechanical and physical properties of the polymer by incorporating different nanoparticles (NPs) into PMMA to act as a kind of reinforcing material.[6]

Nanoparticles have often been used in different forms, such as aluminum oxide (Al_2O_3), zirconium dioxide (ZrO_2), titanium dioxide (TiO_2), silicon dioxide (SiO_2), zinc oxide (ZnO), and silver (Ag).[7] The properties of a polymer nanocomposite are determined by the type, concentration, size, and shape of NPs, as well as their interaction with the polymer matrix.[8]

ZrO_2 NPs are broadly used to improve and reinforce the physical and mechanical properties of PMMA.[9] In addition to its excellent biocompatibility, the PMMA/ZrO_2 nanocomposite is esthetically acceptable, since ZrO_2 is white and less likely to alter its color than other metal oxide NPs.[3] ZrO_2 NPs have good wear and corrosion resistance,[10] thermal stability, high fracture strength, high hardness, and high mechanical resistance.

TiO_2 NPs are increasingly used due to their superior mechanical properties[11] and other features, such as nontoxicity, white color, a low cost, biocompatibility, chemical inactivity, a high refractive index, an antimicrobial effect, corrosion resistance, and high microhardness.[12] According to Alwan and Alameer, adding 3 wt% TiO_2 NPs resulted in an increase in the surface hardness, impact strength and transverse strength values of a heat-cured acrylic resin.[13] Ahmed et al. studied the effect of TiO_2 NPs (1 wt% and 5 wt%) on the impact strength, flexural strength and microhardness of 2 heat-polymerized acrylic resins.[14] In their study, the values of flexural strength declined, whereas the values of microhardness increased through adding TiO_2 NPs.[14] Aziz revealed that impact strength and color stability were improved by the incorporation of 3 wt% TiO_2 NPs; yet, there was no change recorded for thermal conductivity.[15]

The reason behind considering Ag NPs is that they are characterized by distinctive chemical, biological and physical properties, including chemical stability, nonlinear optical behavior, and high electrical and thermal conductivity.[16] Ag NPs show antimicrobial activity against many microorganisms, such as *Streptococcus mutants*, *Candida albicans* and *Staphylococcus aureus*.[17] The reinforcement of PMMA properties, especially the physical and mechanical ones, e.g., compressive strength and thermal conductivity, is attributed to the addition of Ag NPs.[17,18] Mahross and Ebrahim investigated the effect of adding different concentrations of Ag NPs (1 wt%, 2 wt% and 5 wt%) to a heat-cured acrylic resin.[19] The authors found that the incorporation of Ag NPs into the acrylic denture base material improved its color stability, with the greatest effect observed with the 5 wt% concentration, followed by 2 wt% and 1 wt%.[19] However, different problems may occur with regard to the wear of dentures due to the nature of the material itself and continuous use for a long period in a moist environment. Aging fractures, pigment adhesion and color changes are examples of the problems expected.[20]

Due to the features of NPs, impregnating acrylic resins with them may enhance the physical and mechanical properties of the materials. Therefore, the present in vitro study aimed to evaluate the influence of adding ZrO_2, TiO_2 and Ag NPs on the microhardness, abrasive wear resistance and color stability of a heat-cured acrylic resin denture base. The null hypothesis was that ZrO_2, TiO_2, and Ag NPs would not improve the microhardness, abrasive wear resistance and color stability of the heat-cured acrylic resin.

Material and methods

Sample grouping

The materials used in the study and their composition are shown in Table 1. Three types of nanopowders were used at 5 wt%: ZrO_2; TiO_2; and Ag. They were added to the

Table 1. Composition of the materials used for the control and experimental groups

Groups	Composition of the material
Group 1 (control)	heat-cured denture base acrylic resin (methyl methacrylate polymer in a powder form); methyl methacrylate monomer in a liquid form; Cd-free
Group 2	heat-cured denture base acrylic resin (methyl methacrylate polymer in a powder form); methyl methacrylate monomer in a liquid form; Cd-free; 5 wt% ZrO_2
Group 3	heat-cured denture base acrylic resin (methyl methacrylate polymer in a powder form); methyl methacrylate monomer in a liquid form; Cd-free; 5 wt% TiO_2
Group 4	heat-cured denture base acrylic resin (methyl methacrylate polymer in a powder form); methyl methacrylate monomer in a liquid form; Cd-free; 5 wt% Ag

Cd – cadmium; ZrO_2 – zirconium dioxide; TiO_2 – titanium dioxide; Ag – silver.

heat-cured acrylic resin (Superacryl™ Plus; SpofaDental, Jicin, Czech Republic) to form 120 samples divided into 4 groups. Group 1 was represented by pure PMMA without any additive (the control group; n = 30). The other 3 experimental groups were as follows: group 2 was treated with 5 wt% ZrO_2 (purity: 99.9%, nanopowder particle size: 50 nm, MW (molecular weight) = 123.22 g/mol, CAS number 1314-23-4; US Research Nanomaterials, Inc., Houston, USA; n = 30); group 3 was treated with 5 wt% TiO_2 (purity: 99.9%, nanopowder particle size: 50 nm, morphology: near-spherical; US Research Nanomaterials, Inc.; n = 30); and group 4 was treated with 5 wt% Ag (purity: 99.99%, nanopowder particle size: 50 nm, morphology: spherical; US Research Nanomaterials, Inc.; n = 30). Each group was divided into 3 subgroups according to the test performed: the microhardness test; the abrasive wear test; and finally the color stability test.

Sample preparation

According to the ISO 20795-1:2008 standard, the samples were made from metal patterns to get the desired shapes and dimensions. For the microhardness test, the dimensions were 25 mm length × 10 mm width × 3 mm thickness ±0.2 mm.[14] For the abrasive wear test, the sample dimensions were 30 mm length × 10 mm diameter, cylindrical in shape; the dimensions were selected with regard to the abrasive wear test machine used in material engineering science (University of Technology, Baghdad, Iraq). As for the color stability test, 35 mm length × 15 mm width × 0.5 mm thickness dimensions were used according to the American Dental Association (ADA) guidelines.[21] To create the molds, the metal patterns were covered with a separating medium (SpofaDental) and left to dry. They were then embedded into two-part flasks (Hanau Engineering Co., Buffalo, USA), with a dental stone type IV (Zhermack, Badia Polesine, Italy) in the lower part of the flask, mixed according to the manufacturer's instructions. Only a half of the thickness of the metal pattern was put in the dental stone. After the stone completely set, another layer of the separating medium was applied and allowed to dry. Then, the upper part of the flask was used and another portion of dental stone was poured until the material extruded from the flask slot. After that, the flask was put aside for dental stone crystallization. Then, the flask was opened carefully and the metal pattern was removed from the mold.

Addition of nanofillers

The ZrO_2 nanofillers (5 wt%) were added to the resin monomer, and then mixed through the extreme sonication of the fillers. The NPs were suspended in the liquid monomer and well dispersed in the liquid with the use of a sonication probe at 120 V and 60 kHz (Soniprep 150; MSE (UK) Ltd., London, UK), and then separated into individual nanocrystals for 3 min.[15] To prevent the particle aggregation and segregation as much as possible, the liquid monomer of methyl methacrylate (MMA) with ZrO_2 NPs was blended with the acrylic powder instantly. All the proportions and the manipulations of the acrylic resin were in accordance with the manufacturer's instructions. The recommended mixing ratio was 10 mL of liquid monomer and 22 g of powder polymer, which represented a 3:1 volume ratio. The mixture was left aside until it reached the dough stage. According to the ISO 9001 standard, an electronic balance (the management system certified up to an accuracy of 0.0000 g; Denver Instrument, Göttingen, Germany) was used for measuring the weight of the material. For TiO_2 and Ag NPs, the same procedure was followed, whereas the usual procedure was applied for the control group – according to the manufacturer's instructions, but without any additions.

Packing and curing

The mold was painted with a separating medium and the mixture in the dough stage was inserted into the mold to be cured. The metal flask in a conventional brass clamp was placed in a water bath (Talleres Mestraitua, MESTRA®, Txorierri Etorbidea, Biscay, Spain) for curing at 74°C for 90 min, and then the temperature was raised to the boiling point for half an hour under the ADA specification.[21] After that, the metal flask was allowed to cool down at room temperature for half an hour. The complete cooling of the metal flask was followed by deflasking, and finally the specimens were removed. Finishing and polishing were performed for all samples to prepare them for testing.

Testing procedure

Microhardness test

The Vickers microhardness for both the control and experimental groups was measured according to the ISO 868:2003 standard. The Shore durometer with scale D was used as the preferable measuring device for plastic materials, whether semi-rigid or hard ones.[22] The microhardness testing machine (DIN ISO 7619, DIN EN ISO 868, DIN 53505, ASTM D 2240; Elcometer, Aalen, Germany) allowed the determination of surface microhardness. The test load was adjusted to a load of 25 g for 10 s, as required.[23] All tests were performed at room temperature.

The following formula was used (Equation 1):

$$VHN = \frac{L}{d^2} \quad (1)$$

where:

VHN – Vickers microhardness [kg/m^2];

L – load [kg]; and

d – length of the diagonals [mm].

Abrasive wear test

Each sample, before being subjected to the abrasive wear test, was weighed using the electronic balance (Denver Instrument); the measurement was recorded and considered as W1. The abrasive wear test was carried out by repeating sliding contact (900 rpm), with a sliding distance of 6.5 cm for each cycle. It was related to the test machine settings. The wear testing procedure was performed for each specimen under the applied load of 5 N, and the number of cycles that each specimen was subjected to was 2,000.[24] The time each specimen was held stationary in the apparatus with the help of screws was 142 s. Continuous rinsing with demineralized water (73°C) was used during the wear test so that the abraded particles could be removed from the sample surface and the wet environment of the oral cavity could be simulated.[25] After completing the wear test, the specimen was removed from the testing device, cleaned of all debris and left to dry. It was then weighed using the electronic balance to find the difference in weight before and after the 2,000 cycles. The measurement was considered to be W2. Each specimen was then placed again in the testing device for another 5,000 cycles; the same procedure as described for 2,000 cycles was followed, with the time for each sample changed to 322 s. After completing the wear test, the sample was removed, cleaned from all debris and left to dry. It was weighed again with the electronic balance to get the difference in weight before and after the 5,000 cycles. The measurement was regarded as W3. The obtained results were analyzed using the one-way analysis of variance (ANOVA).

Color stability test

The color stability evaluation was done for the control and experimental groups with the use of a double-beam ultraviolet-visible spectrophotometer (T80+ UV/VIS Spectrometer; PG Instruments Ltd., Alma Park, UK). The color measurement was performed with the use of curves of spectral reflectance to obtain diffuse reflectance data at every 5 nm in the range of 400–700 nm. The integrating sphere attachment was used for the measurement. The attachment was installed in the spectrophotometer according to the manufacturer's instructions; the supplied white calibration standard was used in the reference port for the calibration of zero, 100% reflectance, and to obtain data. A special holder was used to hold the sample in the attachment.[19] The results were recorded using the Computer Color Matching (CCM) system to be tabulated for the statistical analysis.

Results

According to ANOVA, the microhardness values showed a highly significant difference ($p < 0.01$) for the specimen groups containing 5 wt% ZrO_2, TiO_2 and Ag NPs (Table 2, Fig. 1). Among the 4 test groups, the highest mean microhardness value was found in the PMMA group containing 5 wt% Ag NPs (82.05 ±3.60 kg/m^2), whereas the control group represented the lowest mean microhardness value (73.65 ±3.04 kg/m^2).

With regard to the abrasive wear test with 2,000 cycles, the mean (*M*), standard deviation (*SD*), standard error (*SE*), minimum (min), and maximum (max) values, as well as *p*-values are summarized in Table 3. There were highly significant differences between all test groups in the mean wear values ($p < 0.01$). The highest mean wear value was related to the control group (0.00150 ±0.00047 g), while the lowest mean wear value was observed in the group with Ag NPs (0.00051 ±0.00023 g), as illustrated in the box and whisker plot (Fig. 2).

As presented in Table 4 and Fig. 3, there were highly significant differences in the mean wear values after 5,000 cycles between all test groups ($p < 0.01$). The highest mean wear value was observed in the control group (0.00400 ±0.00124 g), while the lowest for the Ag NPs group (0.00194 ±0.00030 g).

According to the descriptive data regarding color stability, the PMMA specimens without the addition of NPs had a lower mean value of color change (1.687 ±0.144) as compared to the other 3 groups – the TiO_2 NPs group (1.917 ±0.159), the ZrO_2 NPs group (1.946 ±0.022) and the Ag NPs group, which showed the highest mean color change (2.344 ±0.013) (Table 5, Fig. 4).

Table 2. Mean values [kg/m^2] obtained in the microhardness test for all test groups ($N = 40$)

Groups	*n*	*M* ±*SD*	*SE*	Range		*p*-value ANOVA
				min	max	
Control	10	73.65 ±3.04	0.961	70.0	79.0	0.000**
5 wt% ZrO_2 NPs	10	80.55 ±3.12	0.987	75.0	84.5	
5 wt% TiO_2 NPs	10	79.55 ±3.09	0.976	75.0	84.0	
5 wt% Ag NPs	10	82.05 ±3.60	1.139	77.5	88.0	

M – mean; *SD* – standard deviation; *SE* – standard error; min – minimum; max – maximum; NPs – nanoparticles; ** highly statistically significant ($p < 0.01$).

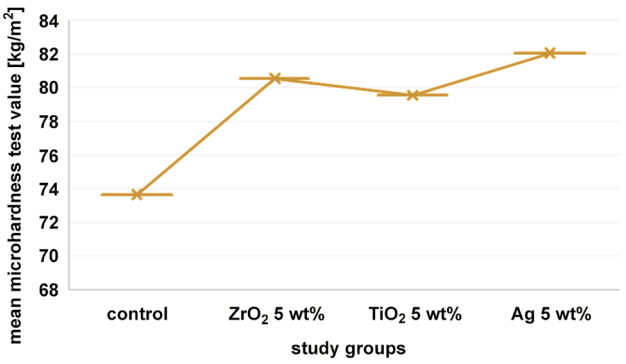

Fig. 1. Distribution of the mean values [kg/m^2] obtained in the microhardness test for all test groups ($N = 40$)

Table 3. Mean values [g] obtained in the abrasive wear test (2,000 cycles – W2) for all test groups ($N = 40$)

Groups	*n*	*M* ±*SD*	*SE*	Range		*p*-value ANOVA
				min	max	
Control	10	0.00150 ±0.00047	0.00015	0.0011	0.0023	0.000**
5 wt% ZrO_2 NPs	10	0.00098 ±0.00049	0.00016	0.0004	0.0019	
5 wt% TiO_2 NPs	10	0.00107 ±0.00043	0.00014	0.0002	0.0019	
5 wt% Ag NPs	10	0.00051 ±0.00023	0.00007	0.0003	0.0009	

** highly statistically significant ($p < 0.01$).

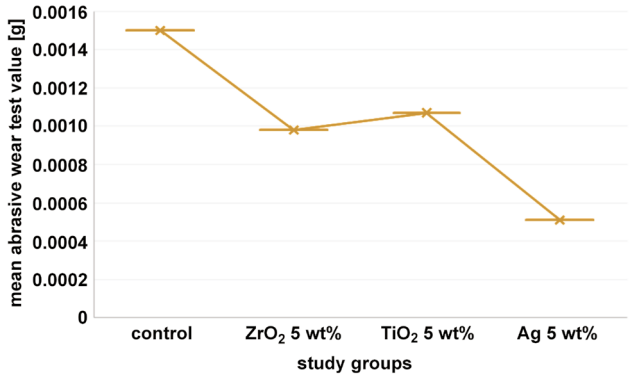

Fig. 2. Distribution of the mean values [g] obtained in the abrasive wear test (2,000 cycles – W2) for all test groups ($N = 40$)

Table 4. Mean values [g] obtained in the abrasive wear test (5,000 cycles – W3) for all test groups ($N = 40$)

Groups	*n*	*M* ±*SD*	*SE*	Range		*p*-value ANOVA
				min	max	
Control	10	0.00400 ±0.00124	0.00039	0.0025	0.0063	0.002**
5 wt% ZrO_2 NPs	10	0.00298 ±0.00155	0.00049	0.0016	0.0068	
5 wt% TiO_2 NPs	10	0.00331 ±0.00080	0.00025	0.0026	0.0055	
5 wt% Ag NPs	10	0.00194 ±0.00030	0.00009	0.0015	0.0024	

** highly statistically significant ($p < 0.01$).

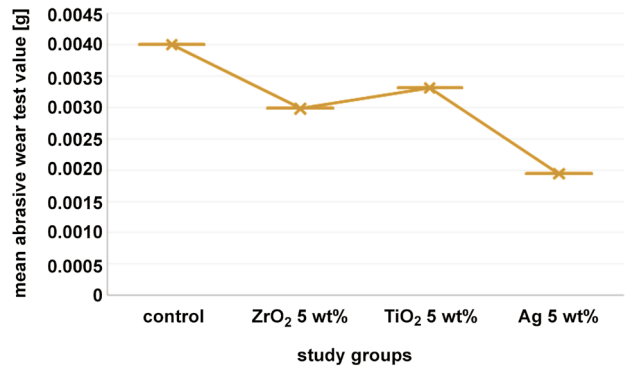

Fig. 3. Distribution of the mean values [g] obtained in the abrasive wear test (5,000 cycles – W3) for all test groups ($N = 40$)

Table 5. Mean values obtained in the color stability test for all test groups ($N = 40$)

Groups	*n*	*M* ±*SD*	*SE*	Range		*p*-value ANOVA
				min	max	
Control	10	1.687 ±0.144	0.083	1.590	1.853	0.001**
5 wt% ZrO_2 NPs	10	1.946 ±0.022	0.013	1.923	1.966	
5 wt% TiO_2 NPs	10	1.917 ±0.159	0.092	1.812	2.100	
5 wt% Ag NPs	10	2.344 ±0.013	0.007	2.331	2.356	

** highly statistically significant ($p < 0.01$).

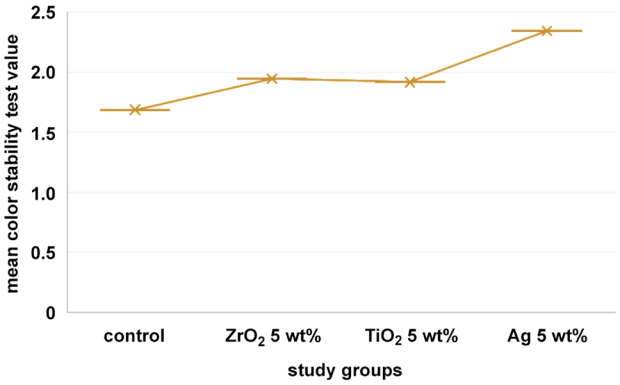

Fig. 4. Distribution of the mean values obtained in the color stability test for all test groups ($N = 40$)

Discussion

There is a sort of agreement between the findings of the present study regarding ZrO_2 NPs and those of Ahmed and Ebrahim, who studied the influence of ZrO_2 NPs at various concentrations (1.5 wt%, 3 wt%, 5 wt%, and 7 wt%) on the fracture toughness, hardness and flexural strength of a heat-polymerized acrylic resin.[26] They observed that the values of hardness had risen in all groups in comparison with control.[26] Besides, the present study is in agreement with the observations made by Hu et al., who evaluated the hardness of PMMA enriched with ZrO_2 NPs at various concentrations (0.5 wt%, 1 wt%, 2 wt%, 3 wt%, 4 wt%, 5 wt%, and 15 wt%) with the use of different tests, e.g., the pendulum hardness and indentation tests.[27] They found that the ratio of ZrO_2 to PMMA was directly proportional to the hardness values, with the highest records being that for 15 wt%.[27] Also, the current study agrees with that of Hameed and Rahman, who used modified zirconia (Zr) at 3 concentrations (3 wt%, 5 wt% and 7 wt%).[28] They found that the cross-linking density was a dominant factor responsible for the increase in nanocomposite hardness at a low NPs concentration level represented by the addition of 3 wt% ZrO_2. On the other hand, the increases in the hardness of the nanocomposite at the 5 wt% and 7 wt% concentrations were highly significant, which could be related to the random distribution of ZrO_2 NPs in the acrylic matrix.[28] Moreover, Zidan et al., who evaluated hardness, fracture toughness,

impact strength, and flexural strength of an acrylic resin, also found that surface hardness continuously increased with increases in the Zr content.[29] However, the findings of other studies contradict the results of the present research. Ihab and Moudhaffar found that the increase in the hardness of nano-ZrO_2/PMMA was non-significant.[30] Ayad et al. revealed that the impact strength, hardness, as well as water solubility of high-impact acrylic resins did not change significantly after reinforcement with the Zr powder for any of the concentrations used (5 wt% and 15 wt%), yet hardness significantly increased in comparison with that of TiO_2–free PMMA.[10]

There are a lot of studies investigating the impact of the addition of TiO_2 NPs on the qualities of PMMA. For example, it was found that the fracture toughness, hardness and flexural strength of PMMA could be improved by adding TiO_2 NPs; an increase in the amount of TiO_2 NPs added to PMMA was related to increases in the above-mentioned parameters.[31] Owing to the strong adhesion between TiO_2 NPs and PMMA, the polymer chain movements are hindered by the dispersion of TiO_2 NPs within the matrix, and thus a better modulus can be attributed to the TiO_2 NPs/PMMA composite material.[32] In contrast, according to some other studies, there are no signs of improving the flexural strength of PMMA through the addition of TiO_2 NPs. This might be related to the clustering of the particles within the resin, resulting in its weakness.[33] Ahmed et al. studied the influence of 2 concentrations of TiO_2 (1 wt% and 5 wt%) on the impact strength, microhardness and flexural strength of 2 kinds of acrylic resin (a high-impact acrylic resin and a normal heat-cured acrylic resin).[14] The results showed that the microhardness values for the conventional resin material were significantly increased by adding 5 wt% TiO_2.[14] Therefore, the results of the current study are supported by those of Ahmed et al.,[14] and also Xia et al., who reported that there were 2 factors behind the increases in surface hardness – a silane coupling agent and the proper filler content – which have the capability of increasing the bonding between the resin matrix and the filler.[34] It is in agreement with the research by Hashem et al., who observed increases in the hardness values reaching 20%, 30%, and 34% with 1 wt%, 2 wt% and 3 wt% NPs, respectively, as compared to pure PMMA.[35] This was fully justified by the increased stiffness of the material due to the presence of rigid particles within the matrix, and additionally to a reduction in the matrix mobility.[35] On the other hand, the above results disagree with other findings concerning the addition of TiO_2 to PMMA. Some authors stated that the mechanical features of PMMA and the flexural strength values could be adversely affected by the incorporation of increasing concentrations of TiO_2 NPs.[36]

There have been debatable results reported on how Ag NPs can influence the mechanical features of denture base resins.[37] According to Casemiro et al., the mechanical qualities of denture base resins may be negatively affected depending on the percentage of added Ag.[38] There has been a lot of argument about the most appropriate concentrations for the addition of a variety of nano-metals to the acrylic resin to get its optimal properties. Zidan et al. explained that the best quantity to improve the distribution of the particles and, at the same time, reduce amalgamation was 5 wt%.[29] In addition, the authors emphasized the fact that tight linking to the resin particles would be promoted by smaller NP sizes, and thus the degradation of NPs could be avoided.[29]

The current study evaluated the effect of 5 wt% TiO_2, ZrO_2 and Ag NPs on the abrasive wear resistance properties of PMMA. The wear of PMMA after 2,000 cycles was assessed (W2). As shown in Table 3, differences between the groups in the abrasive wear test values were highly significant ($p < 0.01$). Reduced wear was noted with NPs in comparison with NP-free PMMA resins, as illustrated in the results. Polymethyl methacrylate has a lot of positive mechanical properties, such as discontinuity deformation, rigidity, hardness, and easy processing, in addition to its esthetic and biological features. On the contrary, there are a lot of drawbacks regarding PMMA, such as oral mucosa irritation, aging, poor resistance to wear and tear, color instability, staining or discoloration, and volume shrinkage after polymerization.[20] Manufacturers have tried more than once to enhance the quality of acrylic resin artificial teeth by adding different substances to the material to improve wear resistance, which would lead to an increase in the longevity of dental prosthetics.[39] Mohammed and Mudhaffar designed and evaluated the addition of modified ZrO_2 NPs in various percentages (2 wt%, 3 wt% and 5 wt%) to heat-cured acrylic resin PMMA material.[40] There were highly significant increases in abrasive wear resistance, fatigue strength and tensile strength with 3 wt% and 5 wt% of nanofillers as compared to pure PMMA material.[40] A reduction in abrasive wear can be explained mainly by the physical properties of ZrO_2; they allow the retaining of a highly smooth surface during the entire wear test, thus changing the wear mechanism from severe abrasion to mild sliding wear. To prevent the severe wear of the material caused by abrasive denture cleansers, food or general functional forces, denture base material must have sufficient abrasive wear resistance.[41] Abrasive wear is reduced by a greater hardness of the denture. As for hardness, the results of the above-mentioned research coincide with what was obtained in the current study – high hardness and a reduction in abrasive wear. Similarly, Ahmed et al. revealed that the addition of 5 wt% TiO_2 NPs increased microhardness, and consequently resulted in higher wear resistance.[14] Moreover, the results of the current study are in agreement with Vojdani et al., who also found that a higher wear resistance of resin material results from an increase in microhardness.[42] It is also in line with Zhang, who studied the influence of TiO_2 NPs at 4 concentrations (1 wt%, 3 wt%, 5 wt%, and 7 wt%) on the tribological behavior of PMMA.[43] The results indicated

that adding TiO_2 NPs increased wear resistance. Furthermore, surfaces in the NP-added groups were concluded to be smoother.[43] Improvement in wear resistance can be achieved through the enhanced mechanical properties, i.e., hardness. In the above-mentioned study, all NP groups had greater abrasive wear resistance than the control group, and the statistical analysis based on the least significant difference (LSD) test showed a significant difference when comparing the control group with the ZrO_2 group and a non-significant difference when comparing the control group with the TiO_2 group. The statistical analysis demonstrated that the difference was highly significant when comparing the control and the Ag NPs groups, but a non-significant difference was noticed between the ZrO_2 NPs and TiO_2 NPs groups. On the other hand, a significant difference was observed between the ZrO_2 NPs and Ag NPs groups, and a highly significant difference between the TiO_2 NPs and Ag NPs groups.[43] The results mentioned above can be explained with regard to the microhardness values obtained in the current study. It appears that the hardness of the material is the exponent of the wear resistance of the prosthesis.[14]

It is worth mentioning that ideally, mechanical properties should be improved by consolidating filler materials without having any side effects on esthetics.[15] ZrO_2 NPs are considered to be less likely to alter esthetics in comparison with other metal oxide NPs, as they are white.[3] According to the National Institute of Standards and Technology, the color change (ΔE) can be clinically acceptable when it is less than 2 units, which is very low.[19] So, the results of the present study are in agreement with those of Ihab et al., who studied the effect of the addition of Zr on the color qualities of PMMA.[44] They did not notice any remarkable changes in color. As ZrO_2 is white and biocompatible, it does not adversely affect the esthetic appearance of the denture base.[45] A variety of studies have shown that the best color protection results are achieved with TiO_2 NPs as compared to other studied NPs. The present results confirm the findings of Andreotti et al., who found that TiO_2 NPs helped in maintaining color stability.[23] Aziz also found that TiO_2 NPs improved color stability.[15] Ahmed et al. reported that a change in the color of acrylic occurred when the TiO_2 NPs concentration exceeded 5 wt%.[14] A great deal of attention has been dedicated to the addition of an Ag compound to the acrylic resin as a measure against odor and bacterial proliferation in the oral cavity, but the results were unfortunately fruitless regarding the color change.[46] Hamedi-Rad et al. studied the influence of adding 5 wt% Ag NPs to PMMA on changes in the tensile strength, compressive strength and thermal conductivity values of PMMA.[17] They found a rise in the values of compressive strength and thermal conductivity, but a decline in the tensile strength values. Besides, they demonstrated a brownish discoloration of the prosthesis based on adding 5 wt% Ag NPs.[17] This goes with the results of the present study. Also, it agrees with the research by Mahroos and Ebrahim, who found that incorporating 5 wt% Ag NPs brought the highest mean color change.[19] In their study, Oei et al. also reported the poor color stability of the Ag NPs/PMMA composite.[47]

Conclusions

According to the methodology applied in this in vitro study and based on the obtained results, taking into account the study limitations, it was concluded that hardness improved in all NP groups and the Ag NPs group presented the best value in the microhardness test for heat-cured PMMA. Also, abrasive wear resistance increased in all NP groups, with the best value in the abrasive wear test noted for the Ag NPs group. The TiO_2 NPs group had the best color stability, whereas the Ag NPs group had the lowest mean color stability.

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

Shurooq Falih Altaie https://orcid.org/0000-0002-8884-101X

References

1. Gungor H, Gundogdu M, Duymus ZY. Investigation of the effect of different polishing techniques on the surface roughness of denture base and repair materials. *J Prosthet Dent*. 2014;112(5):1271–1277. doi:10.1016/j.prosdent.2014.03.023
2. Peyton FA. History of resins in dentistry. *Dent Clin North Am*. 1975;19(2):211–222. PMID:1090459.
3. Gad mm, Al-Thobity AM, Rahoma A, Abualsaud R, Al-Harbi FA, Akhtar S. Reinforcement of PMMA denture base material with a mixture of ZrO_2 nanoparticles and glass fibers. *Int J Dent*. 2019;2019:2489393. doi:10.1155/2019/2489393
4. Hamouda IM, Beyari mm. Addition of glass fibers and titanium dioxide nanoparticles to the acrylic resin denture base material: Comparative study with the conventional and high impact types. *Oral Health Dent Manag*. 2014;13(1):107–112. PMID:24603926.
5. Zappini G, Kammann A, Wachter W. Comparison of fracture tests of denture base materials. *J Prosthet Dent*. 2003;90(6):578–585. doi:10.1016/j.prosdent.2003.09.008
6. Lee JH, El-Fiqi A, Jo JK, et al. Development of long-term antimicrobial poly(methyl methacrylate) by incorporating mesoporous silica nanocarriers. *Dent Mater*. 2016;32(12):1564–1574. doi:10.1016/j.dental.2016.09.001
7. Alhareb AO, Akil HM, Ahmad ZA. Influence of Al_2O_3/Y-TSZ mixture as filler loading on the radiopacity of PMMA denture base composites. *Procedia Chem*. 2016;19:646–650. doi:10.1016/j.proche.2016.03.065

8. Jordan J, Jacob KI, Tannenbaum R, Sharaf MA, Jasiuk L. Experimental trends in polymer nanocomposites – a review. *Mater Sci Eng A*. 2005;393(1–2):1–11. doi:10.1016/j.msea.2004.09.044
9. Gad mm, Fouda SM, Al-Harbi FA, Näpänkangas R, Raustia A. PMMA denture base material enhancement: A review of fiber, filler, and nanofiller addition. *Int J Nanomedicine*. 2017;12:3801–3812. doi:10.2147/IJN.S130722
10. Ayad NM, Badawi MF, Fatah AA. Effect of reinforcement of high-impact acrylic resin with zirconia on some physical and mechanical properties. *Rev Clín Pesq Odontol*. 2008;4(3):145–151. doi:10.7213/AOR.V4I3.23218
11. Elhadiry SS, Yunus N, Ariffin YT. Effect of cavity preparation on the flexural strengths of acrylic resin repairs. *J Appl Oral Sci*. 2010;18(6):546–550. doi:10.1590/S1678-77572010000600003
12. Ghahremani L, Shirkavand S, Akbari F, Sabzikari N. Tensile strength and impact strength of color modified acrylic resin reinforced with titanium dioxide nanoparticles. *J Clin Exp Dent*. 2017;9(5):e661–e665. doi:10.4317/jced.53620
13. Alwan SA, Alameer SS. The effect of the addition of silanized Nano titania fillers on some physical and mechanical properties of heat cured acrylic denture base materials. *J Bagh Coll Dent*. 2015;27(1):86–91. doi:10.12816/0015269
14. Ahmed MA, El-Shennawy M, Althomali YM, Omar AA. Effect of titanium dioxide nano particles incorporation on mechanical and physical properties on two different types of acrylic resin denture base. *World J Nano Sci Eng*. 2016;6(3):111–119. doi:10.4236/wjnse.2016.63011
15. Aziz HK. TiO_2-nanofillers effects on some properties of highly-impact resin using different processing techniques. *Open Dent J*. 2018;12:202–212. doi:10.2174/1874210601812010202
16. Sivakumar I, Arunachalam KS, Sajjan S, Ramaraju AV, Rao B, Kamaraj B. Incorporation of antimicrobial macromolecules in acrylic denture base resins: A research composition and update. *J Prosthodont*. 2014;23(4):284–290. doi:10.1111/jopr.12105
17. Hamedi-Rad F, Ghaffari T, Rezaii F, Ramazani A. Effect of nanosilver on thermal and mechanical properties of acrylic base complete dentures. *J Dent (Tehran)*. 2014;11(5):495–505. PMID:25628675. PMCID:PMC4290768.
18. Ghafari T, Rad FH, Ezzati B. Does addition of silver nanoparticles to denture base resin increase its thermal conductivity? *Journal of Dental School, Shahid Beheshti University of Medical Sciences*. 2014;32(3):139–144. doi:10.22037/jds.v32i3.24789
19. Mahross HZ, Ebrahim MI. Effect of adding nanoparticles of silver on color stability of acrylic resin denture base material. *Egypt Dent J*. 2013;59(4):3939–3944.
20. Wang W, Liao S, Zhu Y, Liu M, Zhao Q, Fu Y. Recent applications of nanomaterials in prosthodontics. *J Nanomater*. 2015;2015:408643. doi:10.1155/2015/408643
21. American Dental Association specification No. 12 for denture base polymer guide to dental materials and devices. 7th ed. 1999. Chicago, USA.
22. Cierech M, Osica I, Kolenda A, et al. Mechanical and physicochemical properties of newly formed ZnO-PMMA nanocomposites for denture bases. *Nanomaterials (Basel)*. 2018;8(5):305. doi:10.3390/nano8050305
23. Andreotti AM, Goiato MC, Moreno A, Nobrega AS, Pesqueira AA, dos Santos DM, Influence of nanoparticles on color stability, microhardness, and flexural strength of acrylic resins specific for ocular prosthesis. *Int J Nanomedicine*. 2014;9:5779–5787. doi:10.2147/IJN.S71533
24. Zarpelon Silva CM, de Paula Eduardo JV, Miranda ME, Basting RT, Novaes Olivieri KA. A method of comparing the wear resistance of various materials used for artificial teeth. *Rev Gaúch Odontol*. 2014;62(3):229–234. doi:10.1590/1981-86372014000300000012170
25. Reis KR, Bonfante G, Pegoraro LF, Rodrigues Conti PC, Garcia de Oliveira PC, Kaizer OB. In vitro wear resistance of three types of polymethyl methacrylate denture teeth. *J Appl Oral Sci*. 2008;16(3):176–180. doi:10.1590/s1678-77572008000300003
26. Ahmed MA, Ebrahim MI. Effect of zirconium oxide nano-fillers addition on the flexural strength, fracture toughness, and hardness of heat-polymerized acrylic resin. *World J Nano Sci Eng*. 2014; 4(2):50–57. doi:10.4236/wjnse.2014.42008
27. Hu Y, Zhou S, Wu L. Surface mechanical properties of transparent poly(methyl methacrylate)/zirconia nanocomposites prepared by in situ bulk polymerization. *Polymer*. 2009;50(15):3609–3616. doi:10.1016/j.polymer.2009.03.028
28. Hameed HK, Rahman HA. The effect of addition nano particle ZrO_2 on some properties of autoclave processed heat cure acrylic denture base material. *J Bagh Coll Dent*. 2015;27(1):32–39. doi:10.12816/0015262
29. Zidan S, Silikas N, Alhotan A, Haider J, Yates J . Investigating the mechanical properties of ZrO_2-impregnated PMMA nanocomposite for denture-based applications. *Materials (Basel)*. 2019;12(8):1344. doi:10.3390/ma12081344
30. Ihab NS, Moudhaffar M. Evaluation the effect of modified nano-fillers addition on some properties of heat cured acrylic denture base material. *J Bagh Coll Dent*. 2011;23(3):23–29.
31. Asar NV, Albayrak H, Korkmaz K, Turkyilmaz I. Influence of various metal oxides on mechanical and physical properties of heat-cured polymethyl methacrylate denture base resins. *J Adv Prosthodont*. 2013;5(3):241–247. doi:10.4047/jap.2013.5.3.241
32. Gad mm, Abualsaud R. Behavior of PMMA denture base materials containing titanium dioxide nanoparticles: A literature review. *Int J Biomater*. 2019;2019:6190610. doi:10.1155/2019/6190610
33. Nejatian T, Johnson A, van Noort R. Reinforcement of denture base resin. *Adv Sci Tech*. 2006;49:124–129. doi:10.4028/www.scientific.net/AST.49.124
34. Xia Y, Zhang F, Xie H, Gu N. Nanoparticle-reinforced resin-based dental composites. *J Dent*. 2008;36(6):450–455. doi:10.1016/j.jdent.2008.03.001
35. Hashem M, Rez MF, Fouad H, et al. Influence of titanium oxide nanoparticles on the physical and thermomechanical behavior of poly methyl methacrylate (PMMA): A denture base resin. *Sci Adv Mater*. 2017;9(6):938–944. doi:10.1166/sam.2017.3087
36. Sodagar A, Bahador A, Khalil S, Shahroudi AS, Kassaee MZ. The effect of TiO_2 and SiO_2 nanoparticles on flexural strength of poly(methyl methacrylate) acrylic resins. *J Prosthodont Res*. 2013;57(1):15–19. doi:10.1016/j.jpor.2012.05.001
37. Gad M, ArRejaie AS, Abdel-Halim MS, Rahoma A. The reinforcement effect of nano-zirconia on the transverse strength of repaired acrylic denture base. *Int J Dent*. 2016;2016.7094056. doi:10.1155/2016/7094056
38. Casemiro LA, Gomes Martins CH, Panzeri Pires-de-Souza FdC, Panzeri H. Antimicrobial and mechanical properties of acrylic resins with incorporated silver-zinc zeolite – part I. *Gerodontology*. 2008;25(3):187–194. doi:10.1111/j.1741-2358.2007.00198.x
39. Stober T, Henninger M, Schmitter M, Pritsch M, Rammelsberg P. Three-body wear of resin denture teeth with and without nanofillers. *J Prosthet Dent*. 2010;103(2):108–117. doi:10.1016/S0022-3913(10)60014-5
40. Mohammed D, Mudhaffar M. Effect of modified zirconium oxide nano-fillers addition on some properties of heat cure acrylic denture base material. *J Bagh Coll Dent*. 2012;24(4):1–7.
41. Ali IL,Yunus N, Abu-Hassan MI. Hardness, flexural strength, and flexural modulus comparisons of three differently cured denture base systems. *J Prosthodont*. 2008;17(7):545–549. doi:10.1111/j.1532-849X.2008.00357.x
42. Vojdani M, Bagheri R, Reza Khaledi AA. Effects of aluminum oxide addition on the flexural strength, surface hardness, and roughness of heat-polymerized acrylic resin. *J Dent Sci*. 2012;7(3):238–244. doi:10.1016/j.jds.2012.05.008
43. Zhang JG. Study on friction and wear behavior of PMMA composites reinforced by HCl-immersed TiO_2 particles. *J Thermoplast Compos Mater*. 2014;27(5):603–610. doi:10.1177/0892705712453153
44. Ihab NS, Hassanen KA, Ali NA. Assessment of zirconium oxide nanofillers incorporation and silanation on impact, tensile strength and color alteration of heat polymerized acrylic resin. *J Bagh Coll Dent*. 2012;24(Special Issue 2):36–42.
45. Sahin Z, Ergun G. The assessment of some physical and mechanical properties of PMMA added different forms of nano-ZrO_2. *J Dent Oral Health*. 2017;3(2):064.
46. Yoshida K, Aoki H, Yoshida T. Color change capacity of dental resin mixed with silver methacrylate caused by light irradiation and heating. *Dent Mater J*. 2009;28(3):324–337. doi:10.4012/dmj.28.324
47. Oei JD, Zhao WW, Chu L, et al. Antimicrobial acrylic materials with in situ generated silver nanoparticles. *J Biomed Mater Res B Appl Biomater*. 2012;100(2):409–415. doi:10.1002/jbm.b.31963

Original paper

Effect of the CAD-CAM and lost-wax framework fabrication techniques on the fracture strength of porcelain in metal-ceramic restorations

Gelareh Tajziehchi[1,A–E], Homeira Ansarilari[2,C,E,F], Kourosh Afshar[2,B,D,E]

[1] Department of Restorative Dentistry, School of Dentistry, Guilan University of Medical Sciences, Rasht, Iran
[2] Department of Prosthodontics, Islamic Azad University, Tehran Dental Branch, Tehran, Iran

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):303–309

Address for correspondence
Kourosh Afshar
E-mail: dr.kouroshafshar@yahoo.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
The authors wish to express their thanks to the late Dr. Seyyed Mostafa Fatemi for his support and helpful advice on several technical issues.

Received on July 31, 2021
Reviewed on January 8, 2022
Accepted on January 29, 2022

Published online on May 29, 2023

Abstract

Background. Ceramic fracture is a common problem in metal-ceramic restorations (MCRs). The advent of computer-aided design and computer-aided manufacturing (CAD-CAM) technology eliminated the lost-wax technique, which was responsible for many of the problems associated with framework fabrication. However, the role of the CAD-CAM technology in decreasing porcelain fracture is not yet known.

Objectives. The aim of the present in vitro study was to compare the fracture strength of porcelain in MCRs with metal frameworks fabricated with the use of the lost-wax and CAD-CAM techniques.

Material and methods. Twenty metal dies were prepared with a deep chamfer finish line, with a depth of 1.2 mm and the occlusal taper of the walls of 8°, a 2-millimeter occlusal reduction of the functional cusp, a 1.5-millimeter occlusal reduction of the nonfunctional cusp, and the functional cusp bevel. Ten frameworks were fabricated using the CAD-CAM system and 10 with the lost-wax technique. After porcelain veneering, the specimens underwent thermocycling and cyclic loading to simulate the aging process. The load test was then performed. The fracture strength of porcelain was compared between the 2 groups, and the mode of failure was also determined using a stereomicroscope.

Results. Two specimens were excluded from the CAD-CAM group. Thus, 18 specimens were statistically analyzed. The results revealed no significant difference in fracture strength between the 2 groups ($p > 0.05$). The mode of failure was mixed in all specimens from both groups.

Conclusions. Our results indicated that the fracture strength of porcelain and the mode of failure did not depend on the metal framework fabrication technique (lost-wax or CAD-CAM).

Keywords: CAD-CAM, fracture strength, Co-Cr, lost-wax, metal-ceramic

Cite as
Tajziehchi G, Ansarilari H, Afshar K. Effect of the CAD-CAM and lost-wax framework fabrication techniques on the fracture strength of porcelain in metal-ceramic restorations. *Dent Med Probl.* 2023;60(2):303–309. doi:10.17219/dmp/146246

DOI
10.17219/dmp/146246

Introduction

Metal-ceramic restorations (MCRs) are still widely used due to their optimal physical properties and a lower cost as compared to all-ceramic restorations.[1–3] Also, MCRs reportedly have a higher 5-year survival rate than all-ceramic restorations.[4] Evidence shows that dental caries, followed by porcelain fracture and chipping are the main causes of failure of MCRs.[5,6] Several strategies have been suggested to prevent porcelain fracture or chipping in MCRs. Modifying the framework design is one of such strategies.[7]

Cobalt-chromium (Co-Cr) alloys are among the most commonly used alloys for the fabrication of MCRs, with successful clinical application since 1930.[8] Cobalt provides hardness, while Cr enhances the physical properties of the alloy and prevents its corrosion. Molybdenum (Mo), which is also present in this alloy, optimizes other particles, creates space during the solidification process and increases the strength of the alloy. It is also responsible for resistance to corrosion. Tungsten (W) has effects similar to those of Mo on the properties of the Co-Cr alloy and is sometimes used as an alternative to Mo.[8] Such alloys are superior to others, e.g., nickel-chromium (Ni-Cr) ones, and have been recommended for the fabrication of dental prosthetic restorations.[9] Frameworks for MCRs can be fabricated with the lost-wax technique, the computer-aided design and computer-aided manufacturing (CAD-CAM) technology, or the laser sintering technique. Among these, the lost-wax technique is most commonly used.[10] This method was first introduced by Dr. B.F. Philbrook in 1897 and gained popularity in 1906.[11] However, problems that may occur during the investment casting and cooling processes in the lost-wax technique are often responsible for the failure of MCRs, since they can compromise the strength of the bond between porcelain and the metal framework.[12,13] Appropriate metal–porcelain bond strength is imperative for porcelain strength.[14] The advent of the CAD-CAM technology eliminated many problems associated with the use of the lost-wax fabrication technique.[7,15–18] The CAD-CAM technology enables the fabrication of restorations with high precision, irrespective of the length of the restoration.[19]

There are different methods of assessing fracture strength, one of which is the load test. During this test, a load is applied vertically to the sample until fracture occurs, and the mode of fracture is subsequently assessed. It can be adhesive, cohesive or mixed. Fracture is considered adhesive when it occurs at the metal–porcelain interface, and it is considered cohesive when it occurs within metal or porcelain. If both adhesive and cohesive types of fracture are detected in the sample, the mode of failure is considered mixed.[18,20–23]

Previous studies evaluated the effect of different methods of fabrication of Co-Cr frameworks on bond strength, yielding controversial results.[20–24] Considering the gap of information, the purpose of the present study was to compare the fracture strength of porcelain in MCRs with Co-Cr frameworks fabricated with the use of the lost-wax technique and the CAD-CAM technology.

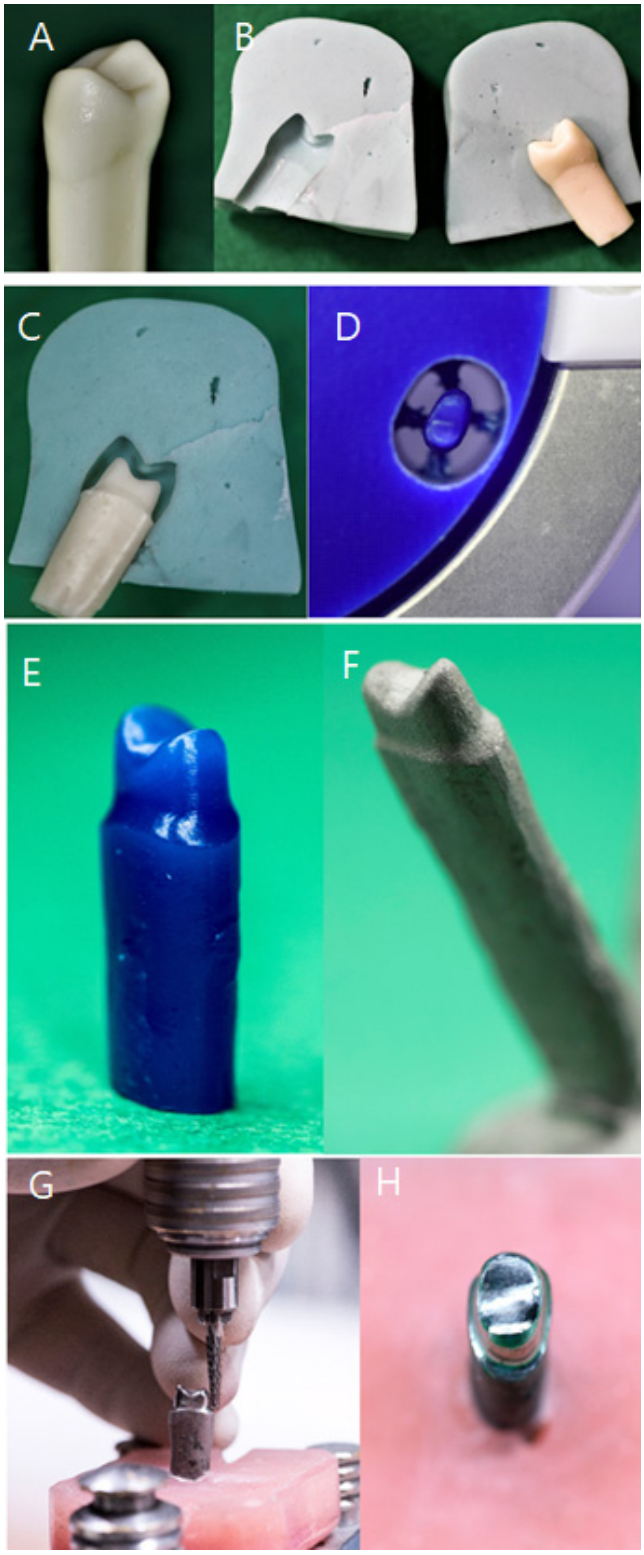

Fig. 1. Cobalt-chromium (Co-Cr) die fabrication

A – maxillary second premolar acrylic tooth; B – putty index prior to the preparation; C – checking the preparation with the use of the index; D – curving the wax block with the use of the computer-aided design and computer-aided manufacturing (CAD-CAM) technology; E – wax pattern of the prepared tooth; F – casting the die; G – modifying the preparation with the use of a surveyor and a dental bur with 4° tapering; H – Co-Cr die.

Material and methods

In this in vitro experimental study, 20 MCRs were fabricated. Ten metal frameworks were fabricated with the lost-wax technique, while another 8 were fabricated using the CAD-CAM technology (2 specimens were excluded).[7,20,25,26] For this purpose, maxillary second premolar acrylic teeth received deep chamfer preparation at the cervical region all-around, with a depth of 1.2 mm, the occlusal taper of the walls of 8°, a 2-millimeter occlusal reduction of the functional cusp, a 1.5-millimeter occlusal reduction of the non-functional cusp, and the functional cusp bevel.[24] Acrylic teeth were then scanned with a Ceramill Map 400 scanner (Amann Girrbach, Pforzheim, Germany) and the information was sent to a Ceramill Motion 2 milling machine (Amann Girrbach). Based on this information, wax blocks (Yamahachi Dental, Gamagori, Japan) were carved and sprued. The metal die was invested with phosphate-bonded investment (Z4 Universal Investment; N&V Belgium, Sint-Niklaas, Belgium) and cast using a Ducatron Quattro casting machine (Ugin Dentaire, Seyssinet-Pariset, France) and the Co-Cr alloy (Magnum Ceramic Co; Mesa Italia, Travagliato, Italy).[15,27,28] The die was then prepared with a tapered bur on a surveyor (Fig. 1). The prepared metal die was scanned and the scan data was transferred to the CAD software in a Ceramic Mind CAD workstation (Amann Girrbach). After assessing the finish line and ensuring that there were no undercuts, the framework was designed with an equal thickness of 0.5 mm, and a die spacer of 50 μ at a 1-millimeter distance from the finish line (Fig. 2).[7,23]

Fabrication of specimens with the lost-wax technique

A CAD-CAM machine was used to increase accuracy, and also for the standardization of specimens. The data regarding the framework design was transferred from the Ceramill Mind CAD workstation to the communicating milling machine (Ceramill Motion 2) and 10 wax patterns were carved out of the wax disks (Fig. 3A,B).[7,24] Next, the specimens were sprued and invested using phosphate-bonded investment (Z4 Universal Investment).

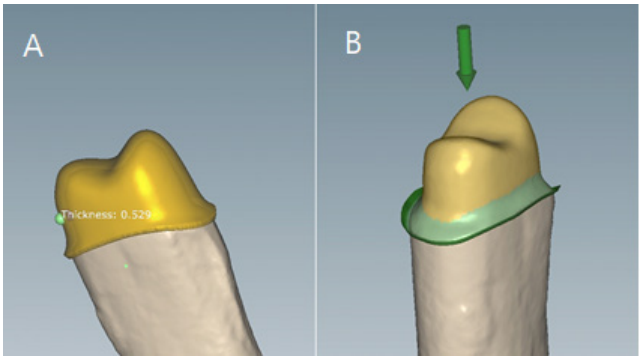

Fig. 2. Die spacer of 50 μ at a 1-millimeter distance from the finish line (A) and the fabrication of a framework with a thickness of 0.5 mm (B)

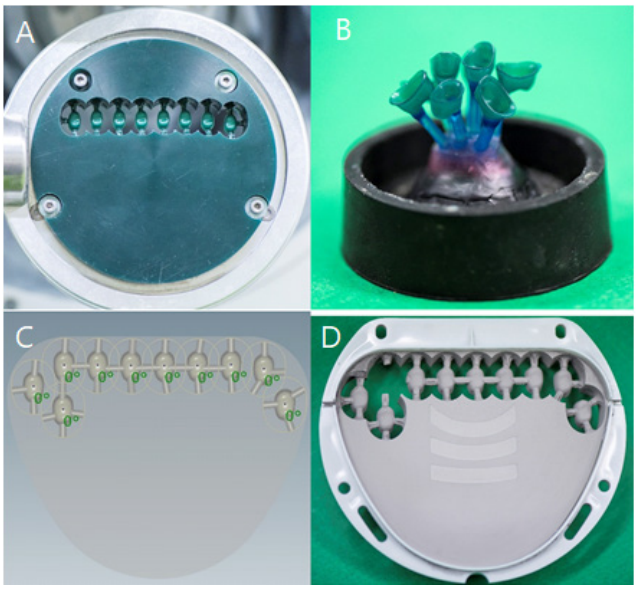

Fig. 3. Framework fabrication with the lost-wax technique (A,B) and the CAD-CAM technique (C,D)

Fabrication of specimens with the CAD-CAM technique

The data regarding the framework design was transferred from the Ceramill Mind CAD workstation to the communicating milling machine (Ceramill Motion 2). Next, 8 specimens were dry-milled by using Co-Cr blocks (Ceramill Sintron blanks; Amann Girrbach) (Fig. 3C,D).[7,20,24] The specimens were then sintered in a Ceramill Argovent 2 sintering compartment (the Ceramill Argotherm 2 system; Amman Girrbach), in an atmosphere of argon gas at 1,280°C for 6 h. The specimens were all homogenous and had no distortions. For surface treatment prior to veneering, according to the manufacturer's instructions, the specimens in the lost-wax group were sandblasted with 150-micrometer aluminum oxide particles, while the specimens in the CAD-CAM group were sandblasted with 50-micrometer aluminum oxide particles for 20 s at an angle of 45° and a distance of 10 mm, under pressure of 3 bars (Basic eco microblaster; Renfert, Hilzingen, Germany). All specimens were subsequently cleaned with 80% ethanol in an ultrasonic bath for 5 min, and then placed in a furnace for oxidation and degaussing (Programat P310; Ivoclar Vivadent, Schaan, Liechtenstein).[7,22,23] The thickness of all specimens was measured with a digital caliper with an accuracy to 0.01 mm. Table 1 shows the information regarding the alloys used in the lost-wax and CAD-CAM techniques.

Porcelain veneering

To standardize the shape and amount of porcelain in all specimens, a full-contour crown was designed using the Ceramill Mind software and carved out of a wax block.

Table 1. Chemical and mechanical properties of the alloys used for the fabrication of frameworks with the lost-wax and CAD-CAM techniques

Properties	Magnum Ceramic Co					Ceramill Sintron				
	Co	Cr	Mo	W	Si, Fe, Mn	Co	Cr	Mo	W	Si, Fe, Mn
	64%	21%	6%	6%	~3%	66%	28%	5%	–	~1%
Yield strength [MPa]	570					450				
Modulus of elasticity [MPa]	194					200				
Elongation at fracture [%]	10					30				
Vickers hardness (HV 10)	286					270				
CTE (25–500°C) [°C^{-1}]	14.1×10^{-6}					14.5×10^{-6}				
Density [g/cm^3]	8.8					7.9				

CTE – coefficient of thermal expansion; Co – cobalt; Cr – chromium; Mo – molybdenum; W – tungsten; Si – silicon; Fe – iron; Mn – manganese.

A putty index (Speedex; Coltène/Whaledent, Altstätten, Switzerland) was made based on the wax model. Two layers of paste (InLine Opaquer A3; Ivoclar Vivadent) were applied to all specimens, and then the porcelain body (InLine Dentin A3; Ivoclar Vivadent) was applied using the silicon index (Fig. 4). Lastly, the specimens were glazed (Fig. 5). One technician performed all the baking procedures according to the manufacturer's instructions (Ivoclar Vivadent).

Simulation of oral conditions

All specimens underwent thermocycling and cyclic loading to simulate normal oral conditions. The specimens were placed in a thermocycler (SD Mechatronik, Feldkirchen-Westerham, Germany) and subjected to 3,000 thermal cycles at 5–55°C. Each cycle lasted 60 s, and included 20 s of dwell time and 20 s of transfer time.[29] For cyclic loading, a chewing simulator (CS-4; SD Mechatronik) was used (Fig. 6A). Using deionized water, 100,000 cycles were applied with a load of 100 N and a frequency of 1 Hz, corresponding to 2–3 months of clinical service.[30,31]

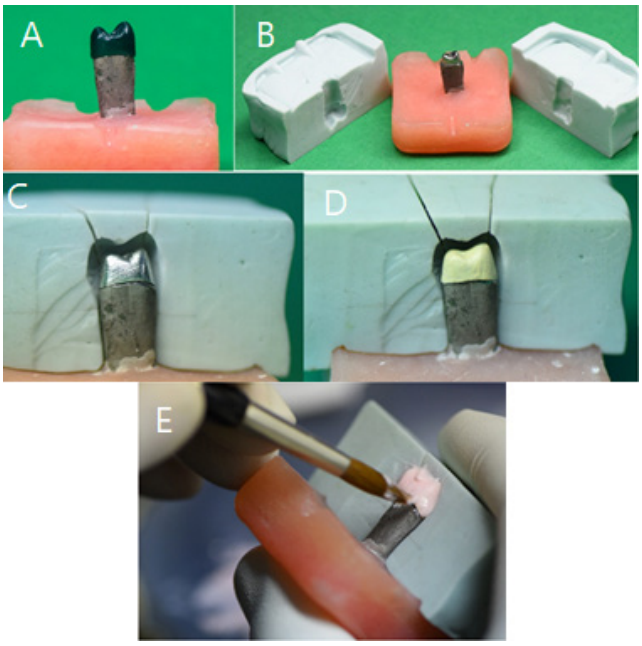

Fig. 4. Porcelain veneering procedure

A – fabrication of a full-contour wax model; B – preparation of a silicone index; C – Co-Cr metal framework; D – placement of a layer of paste on the metal framework; E – placement of the porcelain body, using the silicone index.

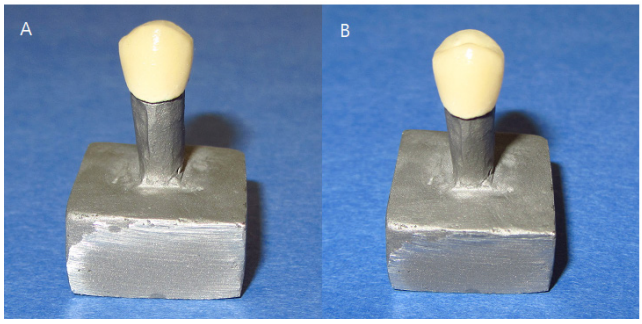

Fig. 5. Specimens after glazing

A – lost-wax group; B – CAD-CAM group.

Load test

The acrylic die stand was first replaced with the Co-Cr alloy to resist forces. The specimen was then cemented on the metal die with the Temp-Bond™ cement (Kerr, Brea, USA). Next, a load was applied to the specimen by means of a round-end stainless steel bar with a diameter of 5 mm at a crosshead speed of 1 mm/min along the tooth longitudinal axis in a universal testing machine (Santam Co., Tehran, Iran) with a capacity of 200 kgf. The load was applied vertically until the fracture of the specimen occurred (Fig. 6B).[28] The load causing fracture was recorded for each specimen and the mode of failure was determined under a stereomicroscope (SMZ800; Nikon, Tokyo, Japan).

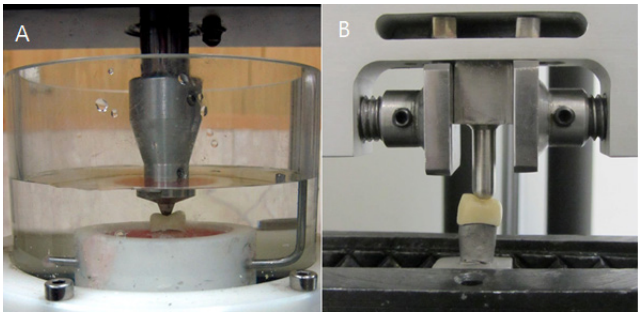

Fig. 6. Simulation of oral conditions and a load test

A – cyclic loading in a CS-4 chewing simulator; B – placement of the specimen in a universal testing machine.

Statistical analysis

The normality of the data was assessed using the Shapiro–Wilk test, and the homogeneity of the data was evaluated using Levene's test. The data was analyzed using IBM SPSS Statistics for Windows, v. 24.0 (IBM Corp., Armonk, USA), and the *t* tests were applied at the significance level of 0.05.

Results

Two specimens were excluded from the CAD-CAM group due to fracture during the study, and 10 specimens in the lost-wax group and 8 specimens in the CAD-CAM group remained in the study. As shown in Table 2, the mean fracture strength was 2,271 ±420 N in the lost-wax group and 2,379 ±531 N in the CAD-CAM group. This difference was not significant ($p > 0.05$). The mode of failure was mixed in all specimens from both groups (Fig. 7).

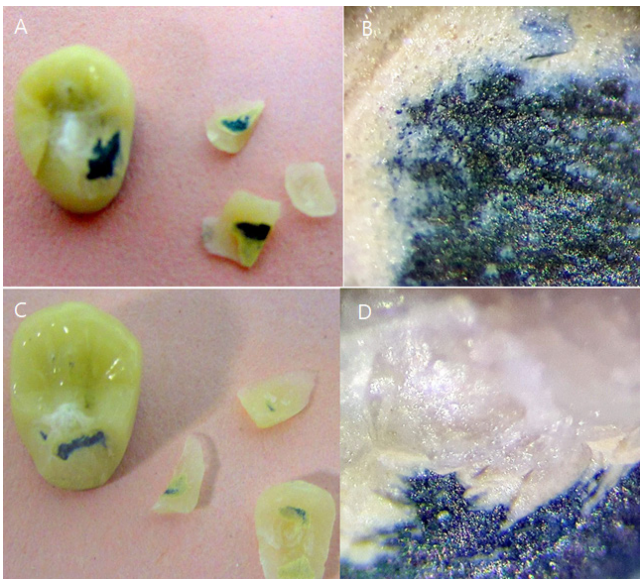

Fig. 7. Fracture surfaces after the load test and the mixed mode of failure

A,B – CAD-CAM group; C,D – lost-wax group.

Table 2. Fracture strength of porcelain in metal-ceramic restorations (MCRs) fabricated with the lost-wax and CAD-CAM techniques, and the mode of failure of the specimens in the 2 groups

Parameter		Lost-wax group $n = 10$	CAD-CAM group $n = 8$
Fracture strength [N] *M* ±*SD*		2,271 ±420	2,379 ±531
Coefficient of variation		18	22
Mode of failure *n*	adhesive	0	0
	cohesive	0	0
	mixed	10	8

M – mean; *SD* – standard deviation.

Discussion

The present study assessed the effect of the lost-wax and CAD-CAM framework fabrication techniques on the fracture strength of porcelain in MCRs. The results showed that the technique of framework fabrication had no significant effect on the fracture strength or the mode of failure of porcelain.

Porcelain chipping/fracture imposes extra costs on patients, and its repair is time-consuming. Both of these factors are clinically important.[25] Thus, adequate metal–ceramic bond strength, metal support for the ceramic material, and/or thickness of the ceramic material are prerequisites in MCRs. Metal–ceramic bond strength depends on many factors, and one of the most influential ones is the metal framework fabrication technique.[15,22]

The composition of the Co-Cr alloy, the porcelain composition, and the difference in the coefficient of thermal expansion (CTE) of the porcelain and the metal are other factors affecting the fracture strength of porcelain.[7,25,32,33] In this study, the CTE of porcelain was 12.9 ±0.5 × 10^{-6}/°C, the CTE of the Magnum Ceramic Co alloy was 14.1 × 10^{-6}/°C and that of the Ceramill Sintron alloy was 14.5 × 10^{-6}/°C; they were all similar. In 2007, Kellerhoff and Fischer measured the fracture strength and thermal shock resistance of MCRs with gold-titanium (Au-Ti) frameworks fabricated with the use of the casting and milling methods.[15] Their results were in contrast to our findings, indicating that the fracture strength of the milling group was significantly lower than that of the casting group. This can be due to the different structure of Au-Ti alloy, since during milling, a soft smear of the Au phase is created on the surface, which serves as a barrier against the diffusion of Ti and prevents the formation of a chemical bond to the ceramic.[15]

No significant difference was noted in the fracture strength of porcelain between the lost-wax and CAD-CAM groups in this study, which may be due to the fact that the specimens had similar oxidation patterns after air abrasion and heat treatment, resulting in similar chemical and mechanical bonding mechanisms. This finding is in agreement with the results of previous

studies.[20,21,23,32,33] Previous studies, however, evaluated rectangular or cylindrical specimens, and most of them did not perform thermocycling and/or cyclic loading. In this study, the specimens had the anatomical form of natural teeth, and underwent both thermocycling and cyclic loading to better simulate the clinical setting.[34] The adopted thermocycling protocol in this study simulated 2.5 years of clinical service,[29] while the cyclic loading protocol simulated 2–3 months of clinical service.[31] Also, water has been suggested to play a role in the propagation of small cracks. Thus, we used deionized water instead of artificial saliva, since it has no significant effect on the coefficient of friction between natural teeth.[7,30] Since MCRs have complex geometries, and the effect of the material properties on fracture strength has not been well elucidated, in vitro studies should preferably simulate the clinical setting as much as possible.[15] Two previous studies used specimens with natural tooth anatomy, performed thermocycling and cyclic loading, and reported results similar to our findings.[7,24] Another study evaluated the properties of the metal–ceramic bond in restorations with Co-Cr frameworks fabricated by means of the casting, milling and selective laser melting (SLM) techniques.[22] They found that metal–ceramic bond strength in the casting group was lower than that in the milling and SLM groups. The variability in the results is likely due to not using specimens with tooth-like anatomy, and not performing thermocycling and cyclic loading.[22]

The mode of failure in this study was mixed for all specimens in both groups. However, cohesive failure within the ceramic was dominant in most mixed fractures. Some previous studies reported that the metal framework fabrication technique had no significant effect on the mode of failure.[15,20,21] Similar to our study, Suleiman and von Steyern did not report any adhesive failure and most fractures were mixed; however, in contrast to our findings, some fractures were purely cohesive in their study.[7] These results may indicate the insignificant effect of debonding forces on the mode of failure.

In the load test, only a vertical load is applied to specimens. Thus, it only simulates vertical load application in the oral environment and misses the loads applied from other directions. Accordingly, the findings of this in vitro study, like many others, cannot be acceptably generalized to the clinical setting. Future clinical trials and prospective in vivo studies are required to more validly elucidate this topic.

Conclusions

Our results indicated that the fracture strength of porcelain and its mode of failure are independent of the metal framework fabrication technique (lost-wax or CAD-CAM). Therefore, both metal framework fabrication techniques can be recommended in 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

Gelareh Tajziehchi https://orcid.org/0000-0003-2518-4283
Homeira Ansarilari https://orcid.org/0000-0001-6916-3869
Kourosh Afshar https://orcid.org/0000-0002-8498-8575

References

1. Kocaağaoğlu H, Kılınç Hİ, Albayrak H, Kara M. In vitro evaluation of marginal, axial, and occlusal discrepancies in metal ceramic restorations produced with new technologies. *J Prosthet Dent*. 2016;116(3):368–374. doi:10.1016/j.prosdent.2016.03.013
2. Suarez MJ, Perez C, Pelaez J, Lopez-Suarez C, Gonzalo E. A randomized clinical trial comparing zirconia and metal-ceramic three-unit posterior fixed partial dentures: A 5-year follow-up. *J Prosthodont*. 2019;28(7):750–756. doi:10.1111/jopr.12952
3. Limones A, Molinero-Mourelle P, Azevedo L, Romeo-Rubio M, Correia A, Gómez-Polo M. Zirconia-ceramic versus metal-ceramic posterior multiunit tooth-supported fixed dental prostheses: A systematic review and meta-analysis of randomized controlled trials. *J Am Dent Assoc*. 2020;151(4):230–238 e7. doi:10.1016/j.adaj.2019.12.013
4. Layton D. A critical appraisal of the survival and complication rates of tooth-supported all-ceramic and metal-ceramic fixed dental prostheses: The application of evidence-based dentistry. *Int J Prosthodont*. 2011;24(5):417–427. PMID:21909482.
5. Behr M, Zeman F, Baitinger T, et al. The clinical performance of porcelain-fused-to-metal precious alloy single crowns: Chipping, recurrent caries, periodontitis, and loss of retention. *Int J Prosthodont*. 2014;27(2):153–160. doi:10.11607/ijp.3440
6. Rosenstiel SF, Land MF, Fujimoto J. *Contemporary Fixed Prosthodontics*. 4th ed. St. Louis, MO: Mosby/Elsevier; 2006:752–768.
7. Suleiman SH, von Steyern PV. Fracture strength of porcelain fused to metal crowns made of cast, milled or laser-sintered cobalt-chromium. *Acta Odontol Scand*. 2013;71(5):1280–1289. doi:10.3109/00016357.2012.757650
8. Al Jabbari YS. Physico-mechanical properties and prosthodontic applications of Co-Cr dental alloys: A review of the literature. *J Adv Prosthodont*. 2014;6(2):138–145. doi:10.4047/jap.2014.6.2.138
9. Loch J, Łukaszczyk A, Augustyn-Pieniążek J, Krawiec H. Electrochemical behaviour of Co-Cr and Ni-Cr dental alloys. *Solid State Phenom*. 2015;227:451–454 doi:10.4028/www.scientific.net/ssp.227.451
10. Shamseddine L, Mortada R, Rifai K, Chidiac JJ. Marginal and internal fit of pressed ceramic crowns made from conventional and computer-aided design and computer-aided manufacturing wax patterns: An in vitro comparison. *J Prosthet Dent*. 2016;116(2):242–248. doi:10.1016/j.prosdent.2015.12.005
11. Shell JS, Nielsen JP. Study of the bond between gold alloys and porcelain. *J Dent Res*. 1962;41:1424–1437. doi:10.1177/00220345620410062101
12. Sadeq A, Cai Z, Woody RD, Miller AW. Effects of interfacial variables on ceramic adherence to cast and machined commercially pure titanium. *J Prosthet Dent*. 2003;90(1):10–17. doi:10.1016/s0022-3913(03)00263-4
13. Vojdani M, Torabi K, Farjood E, Khaledi A. Comparison the marginal and internal fit of metal copings cast from wax patterns fabricated by CAD/CAM and conventional wax up techniques. *J Dent (Shiraz)*. 2013;14(3):118–129. PMID:24724133. PMCID:PMC3927676.

14. Friedlander LD, Munoz CA, Goodacre CJ, Doyle MG, Moore BK. The effect of tooth preparation design on the breaking strength of Dicor crowns: Part 1. *Int J Prosthodont*. 1990;3(2):159–168. PMID:2133383.
15. Kellerhoff RK, Fischer J. In vitro fracture strength and thermal shock resistance of metal-ceramic crowns with cast and machined AuTi frameworks. *J Prosthet Dent*. 2007;97(4):209–215. doi:10.1016/j.prosdent.2007.02.007
16. Hendi A, Falahchai M, Maleki D, Maleki D. Composite preheating. *Journal of Dentomaxillofacial Radiology, Pathology and Surgery*. 2019;8(1):37–40. https://3dj.gums.ac.ir/article-1-464-en.pdf. Accessed July 1, 2021.
17. Padrós R, Punset M, Molmeneu M, et al. Mechanical properties of CoCr dental-prosthesis restorations made by three manufacturing processes. Influence of the microstructure and topography. *Metals*. 2020;10(6):788. doi:10.3390/met10060788
18. Barro Ó, Arias-González F, Lusquiños F, et al. Effect of four manufacturing techniques (casting, laser directed energy deposition, milling and selective laser melting) on microstructural, mechanical and electrochemical properties of Co-Cr dental alloys, before and after PFM firing process. *Metals*. 2020;10(10):1291. doi:10.3390/met10101291
19. Katsoulis J, Mericske-Stern R, Rotkina L, Zbären C, Enkling N, Blatz MB. Precision of fit of implant-supported screw-retained 10-unit computer-aided-designed and computer-aided-manufactured frameworks made from zirconium dioxide and titanium: An in vitro study. *Clin Oral Implants Res*. 2014;25(2):165–174. doi:10.1111/clr.12039
20. Li J, Chen C, Liao J, et al. Bond strengths of porcelain to cobalt-chromium alloys made by casting, milling, and selective laser melting. *J Prosthet Dent*. 2017;118(1):69–75. doi:10.1016/j.prosdent.2016.11.001
21. Serra-Prat J, Cano-Batalla J, Cabratosa-Termes J, Figueras-Àlvarez O. Adhesion of dental porcelain to cast, milled, and laser-sintered cobalt-chromium alloys: Shear bond strength and sensitivity to thermocycling. *J Prosthet Dent*. 2014;112(3):600–605. doi:10.1016/j.prosdent.2014.01.004
22. Wang H, Feng Q, Li N, Xu S. Evaluation of metal-ceramic bond characteristics of three dental Co-Cr alloys prepared with different fabrication techniques. *J Prosthet Dent*. 2016;116(6):916–923. doi:10.1016/j.prosdent.2016.06.002
23. Kaleli N, Saraç D. Comparison of porcelain bond strength of different metal frameworks prepared by using conventional and recently introduced fabrication methods. *J Prosthet Dent*. 2017;118(1):76–82. doi:10.1016/j.prosdent.2016.12.002
24. Krug KP, Knauber AW, Nothdurft FP. Fracture behavior of metal-ceramic fixed dental prostheses with frameworks from cast or a newly developed sintered cobalt-chromium alloy. *Clin Oral Investig*. 2015;19(2):401–411. doi:10.1007/s00784-014-1233-2
25. Hong JT, Shin SY. A comparative study on the bond strength of porcelain to the millingable Pd-Ag alloy. *J Adv Prosthodont*. 2014;6(5):372–378. doi:10.4047/jap.2014.6.5.372
26. Brunner KC, Özcan M. Load bearing capacity and Weibull characteristics of inlay-retained resin-bonded fixed dental prosthesis made of all-ceramic, fiber-reinforced composite and metal-ceramic after cyclic loading. *J Mech Behav Biomed Mater*. 2020;109:103855. doi:10.1016/j.jmbbm.2020.103855
27. Michalakis KX, Stratos A, Hirayama H, Kang K, Touloumi F, Oishi Y. Fracture resistance of metal ceramic restorations with two different margin designs after exposure to masticatory simulation. *J Prosthet Dent*. 2009;102(3):172–178. doi:10.1016/S0022-3913(09)60141-4
28. Urapepon S, Taenguthai P. The effect of zirconia framework design on the failure of all-ceramic crown under static loading. *J Adv Prosthodont*. 2015;7(2):146–150. doi:10.4047/jap.2015.7.2.146
29. Henriques B, Gonçalves S, Soares D, Silva FS. Shear bond strength comparison between conventional porcelain fused to metal and new functionally graded dental restorations after thermal-mechanical cycling. *J Mech Behav Biomed Mater*. 2012;13:194–205. doi:10.1016/j.jmbbm.2012.06.002
30. DeLong R, Douglas WH. An artificial oral environment for testing dental materials. *IEEE Trans Biomed Eng*. 1991;38(4):339–345. doi:10.1109/10.133228
31. Alvarez-Arenal A, Gonzalez-Gonzalez I, deLlanos-Lanchares H, Brizuela-Velasco A, Pinés-Hueso J, Ellakuria-Echebarria J. Retention strength after compressive cyclic loading of five luting agents used in implant-supported prostheses. *Biomed Res Int*. 2016;2016:2107027. doi:10.1155/2016/2107027
32. Stawarczyk B, Eichberger M, Hoffmann R, et al. A novel CAD/CAM base metal compared to conventional CoCrMo alloys: An in-vitro study of the long-term metal-ceramic bond strength. *Oral Health Dent Manag*. 2014;13(2):446–452. PMID:24984663.
33. Choi YJ, Koak JY, Heo SJ, Kim SK, Ahn JS, Park DS. Comparison of the mechanical properties and microstructures of fractured surface for Co-Cr alloy fabricated by conventional cast, 3-D printing laser-sintered and CAD/CAM milled techniques. *J Korean Acad Prosthodont*. 2014;52(2):67–73. doi:10.4047/jkap.2014.52.2.67.
34. Lopez-Suarez C, Tobar C, Sola-Ruiz MF, Pelaez J, Suarez MJ. Effect of thermomechanical and static loading on the load to fracture of metal-ceramic, monolithic, and veneered zirconia posterior fixed partial dentures. *J Prosthodont*. 2019;28(2):171–178. doi:10.1111/jopr.13008

Original paper

Effect of the application of a hydrogen peroxide home bleaching agent on the cytotoxicity of different CAD-CAM restorative materials

Cenk Serhan Ozverel[1,2,A–D], Sevcan Kurtulmus-Yilmaz[3,A,D–F]

[1] Department of Basic Medical Sciences, Faculty of Dentistry, Near East University, Nicosia, Cyprus
[2] DESAM Institute, Near East University, Nicosia, Cyprus
[3] Department of Prosthodontics, Faculty of Dentistry, Near East University, Nicosia, Cyprus

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):311–320

Address for correspondence
Sevcan Kurtulmus-Yilmaz
E-mail: sevcankurtulmusyilmaz@gmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on May 26, 2021
Reviewed on September 26, 2021
Accepted on October 1, 2021

Published online on June 5, 2023

Abstract

Background. The information regarding the cytotoxicity of ceramic and resin-matrix ceramic materials subjected to over-the-counter bleaching agents is limited in the literature.

Objectives. The aim of the present study was to investigate the cytotoxic effects of lithium disilicate ceramic (LDC), resin nano-ceramic (RNC) and nano-hybrid composite (NHC) computer-aided design/computer-aided manufacturing (CAD-CAM) block materials subjected to a home bleaching agent and artificial saliva.

Material and methods. A total of 432 specimens were prepared from 3 different CAD-CAM materials. Each material group was divided into 4 groups according to the storage medium (phosphate-buffered saline (PBS) or artificial saliva), and whether the specimens were subjected to a bleaching agent or not. For the bleached groups, hydrogen peroxide (10%) was applied to the specimens for 30 min/day for 15 days, and the specimens were immersed in PBS or saliva after bleaching. The viability of epithelial cells was detected using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at the end of the 5th, 10th and 15th day of the study. The data was statistically analyzed.

Results. Regardless of the storage medium and the time period, all restorative materials decreased the viability of cells. The highest cytotoxicity levels were determined on the 15th day of the study. The application of a bleaching agent increased the cytotoxicity of the LDC specimens stored in artificial saliva. The RNC material stored in PBS demonstrated significantly higher cell viability than the LDC and NHC groups. The LDC and RNC specimens stored in artificial saliva did not show any significant difference in cytotoxicity. When the materials were subjected to bleaching, NHC demonstrated the highest cytotoxicity during all periods. No significant difference was found between the LDC and RNC specimens subjected to both artificial saliva and bleaching in terms of cytotoxicity.

Conclusions. The type of restorative material, the immersion medium, the application of a bleaching agent, and the application period affected the cytotoxicity of the materials. Over-the-counter home bleaching agents may induce cellular cytotoxicity due to the existing restorations, and patients should be informed about this potential biological response.

Keywords: cytotoxicity, lithium disilicate, home bleaching, resin nano-ceramic, nano-hybrid composite

Cite as
Ozverel CS, Kurtulmus-Yilmaz S. Effect of the application of a hydrogen peroxide home bleaching agent on the cytotoxicity of different CAD-CAM restorative materials. *Dent Med Probl.* 2023;60(2):311–320. doi:10.17219/dmp/142761

DOI
10.17219/dmp/142761

Introduction

In the last decade, the dental industry has focused on the development of new materials with improved optical properties due to the increased demands and expectations of patients regarding esthetic appearance. Computer-aided design/computer-aided manufacturing (CAD-CAM) systems provide the standardized and controlled milling of different types of restorative materials. Ceramics, resin composites, resin-matrix ceramics, and polymethyl methacrylate (PMMA) are indirect restorative materials available as pre-processed blocks. Precisely fitting restorations can be fabricated via the CAD-CAM technology, using these homogenous and defect-free blocks.[1] Among the aforementioned materials, resin-matrix ceramics are relatively new; they have become popular in the last few years. Ceramics show improved optical characteristics, higher biocompatibility, stain resistance, and durability in comparison with resin composites. However, resin composites provide lower abrasion on the antagonist enamel or restorative material, a lower modulus of elasticity, and better polish and repair properties than ceramics. Moreover, the lower brittleness and chipping fracture incidence of resin composites are advantageous when the material is subjected to milling. Therefore, resin-matrix ceramics, which are aimed to combine the beneficial properties of ceramics and composites, are preferred for chairside CAD-CAM restorations.[2] Lava™ Ultimate is the first material introduced as a resin nano-ceramic (RNC) containing silica (Si) and zirconia (Zr) nanoparticles (80 wt%) embedded in a highly cross-linked polymer matrix (20 wt%) composed of bisphenol A glycidyl methacrylate (BisGMA), urethane dimethacrylate (UDMA), ethoxylated bisphenol A dimethacrylate (BisEMA), and triethylene glycol dimethacrylate (TEGDMA).[3]

In previous studies, the mechanical and optical behavior of resin-matrix ceramics was investigated for a better understanding of their clinical performance, and these materials were compared to ceramics and conventional resin composites.[3–7] The mechanical strength of resin-matrix ceramic blocks was reported to be superior to conventional composites,[8] while the flexural properties were found to be comparable to glass ceramic, but inferior to lithium disilicate ceramic (LDC) blocks.[9] Therefore, the properties of resin-matrix materials were considered to be between those of ceramics and conventional resin composites.[10]

At-home and in-office tooth bleaching are widely used procedures to improve the esthetic appearance by eliminating the discoloration of teeth.[11] Higher concentrations of hydrogen peroxide are used during in-office bleaching under the observation of the clinician, while at-home bleaching is performed by the patient with lower concentrations of carbamide peroxide or hydrogen peroxide.[12,13] During these procedures, not only the surfaces of the teeth, but also the existing restorations are subjected to bleaching agents.[14–16] In this sense, the effects of these bleaching agents on the optical properties and surface characteristics of ceramics and resin composites were investigated in several studies,[15–19] and the influence of the bleaching procedures was concluded to be material-dependent.[20] One of the previous studies reported that the bleaching procedures with high-concentration agents increased the surface roughness of RNC materials.[21] Such surface alterations may result from water absorption or the loss of inorganic filler particles, caused by the diffusion of the free radicals released from peroxides into the resin matrix.[22] It has been documented that bleaching increases the release of monomers from resin composites,[23,24] which may also change the surface topography. Since these monomers are reported to be cytotoxic,[25] and as they can be released into the saliva after bleaching and contact oral tissues, the behavior of restorative materials that are subjected to bleaching should be known. Therefore, the present study aimed to investigate the cytotoxicity effects of a home bleaching agent (10% hydrogen peroxide) applied to LDC, RNC and nano-hybrid composite (NHC) CAD-CAM blocks in contact with epithelial cells. The null hypotheses of the study were as follows: (1) storage in artificial saliva and (2) the application of a bleaching agent would not affect the viability of the cells in contact with the restorative materials; (3) there would be no significant differences in the cytotoxicity levels of the restorative materials; (4) the duration of the storage of the restorative materials in artificial saliva and (5) the duration of bleaching agent application would not affect the cytotoxicity of the tested materials.

Material and methods

The study design and test procedures are presented as a flowchart in Fig. 1 and are schematically illustrated in Fig. 2.

Specimen preparation

Specimens of LDC, RNC and NHC of the same shade (A2) were evaluated. The composition and manufacturers of the CAD-CAM restorative materials are presented in Table 1. Thirty-six specimens of a rectangular shape and a thickness of 1.2 mm were obtained from each material (a total number of 108 specimens), using CAD-CAM blocks and a low-speed diamond saw (IsoMet™; Buehler, Lake Bluff, USA) with water cooling. By using a diamond disk (Sunshine Diamond; Dr. Hopf GmbH & Co. KG, Langenhagen, Germany), each specimen was sectioned into 4 equal parts with dimensions of 6 mm × 7 mm × 1.2 mm. Thus, a total of 432 specimens were prepared. The LDC specimens underwent

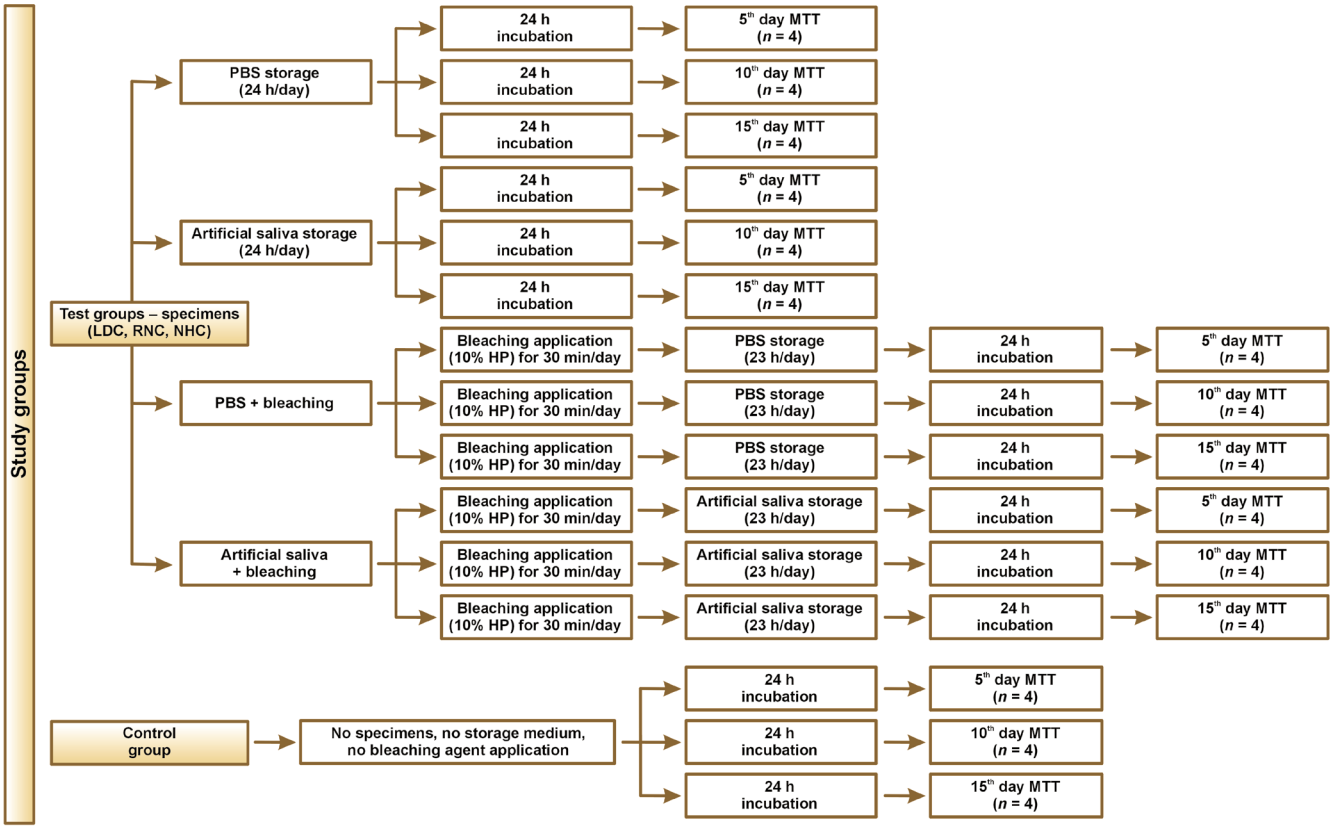

Fig. 1. Flowchart of the study

LDC – lithium disilicate ceramic; RNC – resin nano-ceramic; NHC – nano-hybrid composite; PBS – phosphate-buffered saline; HP – hydrogen peroxide; MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

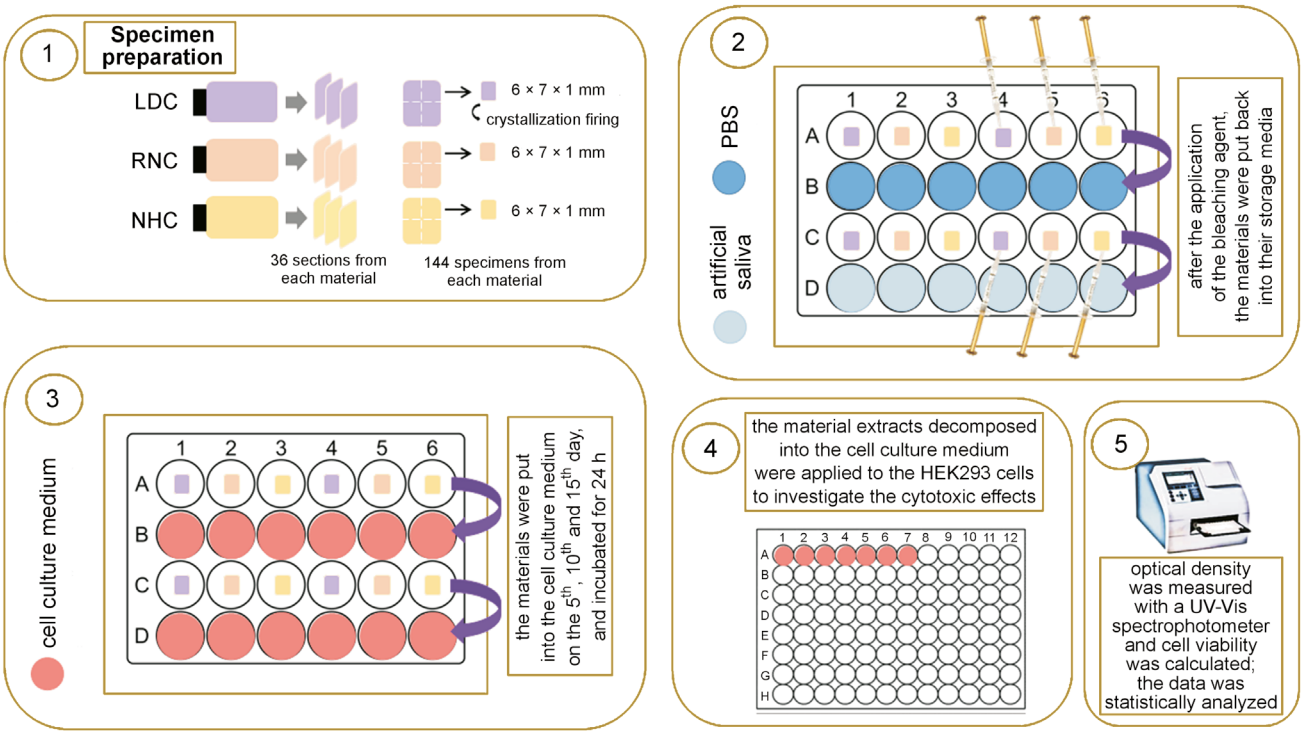

Fig. 2. Schematic illustration of the study design

HEK293 cells – human embryonic kidney epithelial cells; UV-Vis – ultraviolet-visible.

Table 1. Materials evaluated in the study

Material	Type	Composition	Manufacturer
IPS e.max CAD	lithium disilicate glass ceramic	lithium disilicate-reinforced glass ceramic of the Li_2O–K_2O–P_2O_5–MgO material system, ZnO, ZrO_2, Al_2O_3	Ivoclar Vivadent, Schaan, Liechteinstein
Lava™ Ultimate	resin nano-ceramic	20 wt% composite resin material (BisGMA, UDMA, BisEMA, TEGDMA) with 80 wt% Si and Zr nanoparticles and Zr/Si nanoclusters	3M ESPE, Seefeld, Germany
Tetric® CAD	nano-hybrid composite	composite resin material (BisGMA, BisEMA, TEGDMA, UDMA) with 71.1 wt% Ba glass and Si fillers	Ivoclar Vivadent, Schaan, Liechteinstein

BisGMA – bisphenol A glycidyl methacrylate; UDMA – urethane dimethacrylate; BisEMA – ethoxylated bisphenol A dimethacrylate; TEGDMA – triethylene glycol dimethacrylate.

crystallization firing (Programat EP5000; Ivoclar Vivadent, Schaan, Liechtenstein) according to the manufacturer's instructions. All the surfaces of the specimens were ground and polished using under water irrigation with wet silicon carbide papers, following a sequence of 500, 1,200, 2,000, and 4,000 grit to achieve a thickness of 1 mm. The dimensions of the specimens were controlled with a digital caliper (N48AA; Maplin, Rotherham, UK). The specimens were divided into 4 equal groups for each material according to the applied test protocol, as displayed in Table 2. Each group of specimens was further divided into 3 equal subgroups according to the test period (the 5th, 10th and 15th day; n = 4). The specimens in groups 1–6 were not subjected to bleaching, and were stored in either phosphate-buffered saline (PBS) (groups 1–3) or artificial saliva (1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130 mM KCl, 1 mM NaN_3, and 20 mmol/L HEPES) (groups 4–6) during the testing procedures (Table 2).

Application of a bleaching agent

The whole process was carried out in a cell culture cabin (Class II) in a sterile environment and all specimens were autoclaved with a conventional glassware protocol at 121°C for 20 min for sterilization prior to the bleaching procedures. An over-the-counter and prefilled tray-type home bleaching system (Opalescence Go; Ultradent Products Inc., South Jordan, USA) containing 10% hydrogen peroxide was used in this study. A syringe was used to apply an equal amount of the agent on one surface of the specimens and a sterile Heidemann spatula was used to spread the agent uniformly on the surface. According to the protocol recommended by the manufacturer, the application period was 30 min per day. After the bleaching procedure was terminated, the specimens were cleaned and rinsed with PBS. Afterward, the specimens were either put into PBS (groups 7–9) or artificial saliva (groups 10–12) (Table 2). This procedure was repeated for 15 days. At the end of each time period (the 5th, 10th and 15th day), the specimens in their related period subgroups were put into the prepared complete cell culture medium and incubated for another 24 h. Then, the cell culture medium was collected and used for the cytotoxicity study.

Table 2. Test groups according to the restorative material and the test medium

Group No.	Applied protocol
1	PBS + LDC
2	PBS + RNC
3	PBS + NHC
4	artificial saliva + LDC
5	artificial saliva + RNC
6	artificial saliva + NHC
7	PBS + LDC + bleaching agent
8	PBS + RNC + bleaching agent
9	PBS + NHC + bleaching agent
10	artificial saliva + LDC + bleaching agent
11	artificial saliva + RNC + bleaching agent
12	artificial saliva + NHC + bleaching agent

There were also control groups consisting of cells that were not in contact with any restorative material, storage medium or bleaching agent; these cells were incubated during the test period. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Acros Organics, Morris Plains, USA) was performed in the control groups at the end of the 5th, 10th and 15th day.

Cultivation of HEK293 cells

Previously cryopreserved HEK293 human embryonic kidney epithelial cells were thawed in a 37°C water bath and transferred into Falcon® tubes for centrifugation at 1,000 rpm for 5 min. After the centrifugation step, the cells were transferred to 75 cm^3 flasks with a medium consisting of Dulbecco's Modified Eagle Medium/F12 (DMEM/F12) (Gibco™, Visp, Switzerland), supplemented with 10% fetal bovine serum (FBS) (Gibco), 2 mM glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin (Gibco). The cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 and subcultured every 2 days. After reaching 80% confluence, the cells were trypsinized with trypsin-EDTA (Capricorn Scientific, Ebsdorfergrund, Germany) for 10 min at 37°C and 5% CO_2 before being used in the cytotoxicity assay. The cells from passage 3 were used in the study.

In vitro cytotoxicity assay

Cytotoxicity was determined by performing the MTT assay to investigate the activity of mitochondrial enzymes in viable cells. Viable cells can successfully cleave MTT and form formazan crystals with the help of a cellular enzyme succinate dehydrogenase (SDH). The formed formazan crystals are later dissolved by dimethyl sulfoxide (DMSO) to terminate the MTT assay. For this purpose, the cells were trypsinized and seeded into 96-well plates at a density of 2×10^5 cells/well, and incubated for 24 h at 37°C and 5% CO_2. Then, the culture medium was replaced with the medium subjected to a 24-hour incubation period with specimens that were treated differently (Table 2). The cells were incubated with the replaced culture medium for another 24 h before the termination of the study. After 24 h, the MTT solution (1 mg/mL) was added to the cells and they were incubated for 4 h. The images of the cells were taken at the end of the study by using an inverted microscope (Olympus, Tokyo, Japan). The crystals formed by viable cells were dissolved by the addition of 50 µL of DMSO to each well during the post-incubation period. All the different groups were studied in quadruplicate. An ultraviolet-visible (UV-Vis) spectrophotometer (wavelength λ = 570, Multiskan® Spectrum; Thermo Scientific, Waltham, USA) was used to measure the optical density of the dissolved material. The cellular cytotoxicity rate [%] was determined with the following formula (Equation 1):

$$\%\ \text{cytotoxicity (viability)} = \frac{(\text{absorbance of individual group}) - (\text{absorbance of blank})}{(\text{absorbance of control group}) - (\text{absorbance of blank})} \quad (1)$$

Statistical analysis

The data was analyzed with the standard error of the mean (*SEM*) method, and the statistical significance of differences was examined using the one-way analysis of variance (ANOVA) and Tukey's post-hoc tests for multiple intragroup comparisons. Comparisons of groups at different time intervals (on the 5th, 10th and 15th day) were performed via conducting the multiple ANOVA with the use of GraphPad Prism, v. 5.0 (https://www.graphpad.com), and IBM SPSS Statistics for Windows, v. 20.0 (IBM Corp., Armonk, USA). The *p*-values of less than 0.05 were stated as statistically significant.

Results

Regardless of the storage medium and the time period, all test groups showed significantly higher cytotoxicity than the control group ($p < 0.05$) except for RNC immersed in PBS ($p > 0.05$), which showed the lowest cytotoxicity level among all test groups for all time intervals. The lowest cell viability was detected in the NHC groups subjected to PBS/artificial saliva and the bleaching agent for 15 days (Fig. 3).

Within the LDC groups, no significant differences were found among the PBS, artificial saliva and PBS + bleaching groups ($p > 0.05$), while the artificial saliva + bleaching group demonstrated significantly lower cell viability for

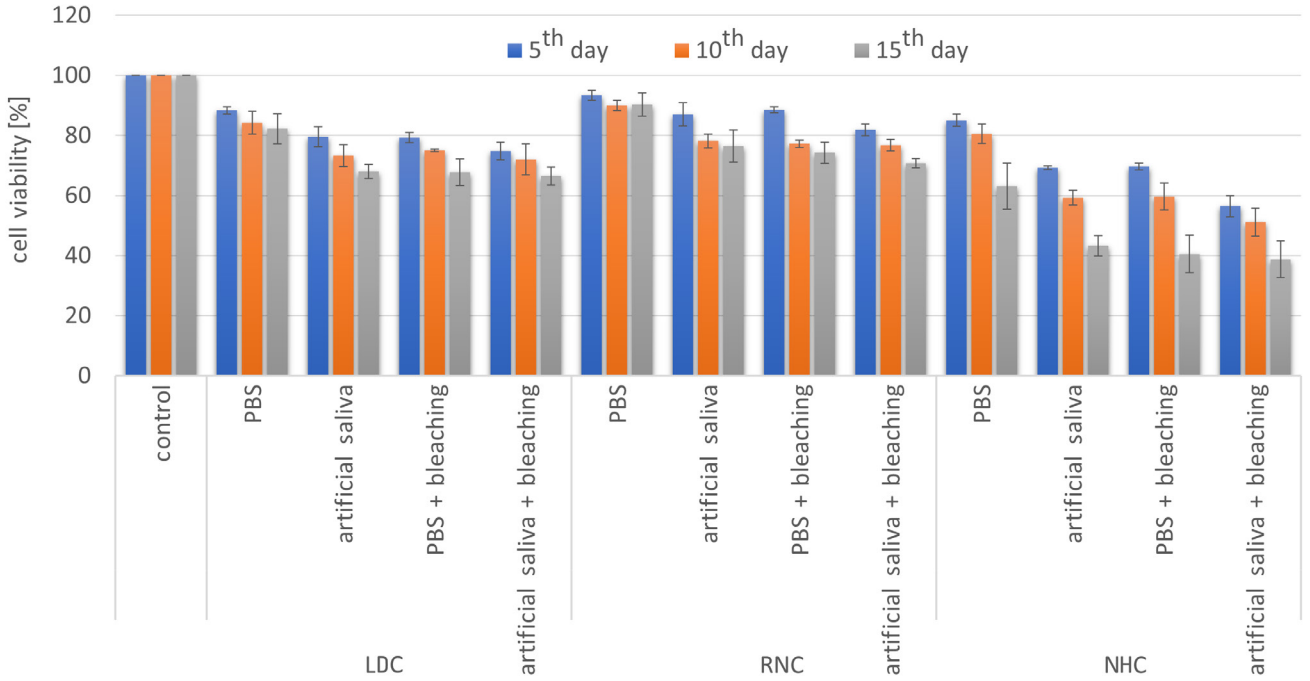

Fig. 3. Cell viability [%] with regard to different computer-aided design/computer-aided manufacturing (CAD-CAM) materials, test media and time periods

all periods ($p < 0.05$). When the periods were compared, no significant differences were detected between the 5th and 10th day; however, cell viability at the end of the 15th day was significantly lower than that of the other periods ($p < 0.05$) for all LDC groups except for the PBS group, where the period parameter did not affect cell viability (Table 3).

The RNC specimens stored in PBS showed significantly lower cytotoxicity as compared to the other RNC groups ($p < 0.05$), which showed similar cell viability for all periods ($p > 0.05$). When the periods were compared for the RNC material, significant differences were detected between the 5th and 15th day for all test groups except for the PBS group (Table 3).

For the NHC material, the PBS group demonstrated remarkably higher cell viability than the other test groups for all periods ($p < 0.05$), whereas there were no significant differences among the other groups ($p > 0.05$). Period comparisons revealed that cell viability at the end of the 5th and 10th day was not statistically different ($p > 0.05$), while the 15th day data showed significantly lower cell viability for all test groups ($p < 0.05$) (Table 3).

The restorative materials were compared with regard to each storage medium and the presence or absence of bleaching to investigate the cytotoxicity of materials subjected to the same protocol. The RNC material stored in PBS showed significantly higher cell viability than the LDC and NHC materials for all periods ($p < 0.05$). No significant differences were found between LDC and RNC stored in artificial saliva ($p > 0.05$); however, significantly lower cell viability percentages were obtained for the NHC material regardless of the period ($p < 0.05$). When the materials were subjected to a bleaching agent and stored in PBS, the cytotoxicity induced by NHC was significantly the highest ($p < 0.05$), while significantly the lowest values were obtained for RNC ($p < 0.05$). On the other hand, the LDC and RNC specimens bleached and stored in artificial saliva did not show statistically significant differences in terms of cell viability ($p > 0.05$). However, NHC demonstrated significantly lower cell viability for all periods ($p < 0.05$) (Table 3).

Discussion

According to the statistical analysis of the data, all the restorative materials subjected to artificial saliva revealed lower cell viability in comparison with the control group, and thus the 1st null hypothesis of the study was rejected. The 2nd null hypothesis of the study was partially accepted, since only the LDC specimens subjected to bleaching and stored in artificial saliva demonstrated significantly lower

Table 3. Viability [%] of the HEK293 cells at different time periods

Material	Test group	5th day	Sig.	10th day	Sig.	15th day	Sig.
–	control	100 ±0	A,a	100 ±0	A,a	100 ±0	A,a
LDC	PBS	88.31 ±1.23	A,b, †	84.23 ±3.80	A,b, †	82.23 ±5.01	A,b, †
	artificial saliva	79.54 ±3.30	A,b, †	73.27 ±3.70	A,b, †	68.01 ±2.39	B,b, †
	PBS + bleaching	79.33 ±1.71	A,b, †	75.01 ±0.45	A,b, †	67.74 ±4.39	B,b,d, †
	artificial saliva + bleaching	74.78 ±2.92	A,c, †	71.98 ±5.13	A,c, †	66.49 ±2.98	B,c,d, †
RNC	PBS	93.34 ±1.58	A,a,d, ‡	89.97 ±1.73	A,a, ‡	90.25 ±3.86	A,a, ‡
	artificial saliva	87.04 ±3.83	A,d, †	78.13 ±2.34	A,B,d, †	76.43 ±5.35	B,e, †
	PBS + bleaching	88.52 ±1.05	A,d, ‡	77.20 ±1.23	A,B,d, ‡	74.22 ±3.56	B,e, ‡
	artificial saliva + bleaching	81.90 ±1.99	A,d, †	76.74 ±1.92	A,B,d, †	70.74 ±1.59	B,e, †
NHC	PBS	85.01 ±2.04	A,e, †	80.51 ±3.26	A,e, †	63.09 ±7.66	B,f, †
	artificial saliva	69.21 ±0.61	A,f, ‡	59.23 ±2.42	A,f, ‡	43.29 ±3.41	B,g, ‡
	PBS + bleaching	69.63 ±1.16	A,f, #	59.65 ±4.45	A,f, #	40.49 ±6.23	B,g, #
	artificial saliva + bleaching	56.42 ±3.58	A,f, ‡	51.15 ±4.60	A,f, ‡	38.74 ±6.08	B,g, ‡

Sig. – statistical significance; the same capital letters in the same row and the same lowercase letters in the same column show no statistically significant difference ($p > 0.05$); the same symbols (†, ‡, #) within the same test group (with regard to the applied protocol) show no statistically significant difference between the restorative materials ($p > 0.05$).

cell viability than the control group and the specimens from the other LDC groups. The 3rd null hypothesis was rejected, since significant differences in cytotoxicity were detected between the restorative materials, depending on the storage medium and the application of a bleaching agent. Immersing the bleached and non-bleached restorative materials in artificial saliva for different periods of time affected cell viability; therefore, the 4th and 5th null hypotheses were also rejected.

At-home bleaching systems can be categorized as professionally supervised and over-the-counter whitening products.[26] The former ones include dentist supervision and provide more controlled application with the use of customized whitening trays.[27] Over-the-counter systems do not require dentist supervision and are preferred by patients[26] due to a shorter application time.[28] The trays used for over-the-counter systems are not customized, and thus the tray cannot fully adapt to the dental arch and provide adequate sealing. This may cause the overflow of the bleaching agent into the oral cavity and its contact with tissues.[29] Therefore, the impact of this type of bleaching kits on the biological responses of intraoral tissues should be evaluated.

Besides assessing the mechanical and optical properties of restorative materials, the in vitro determination of their cytotoxicity is a very crucial step in investigating the occurrence of hazards and any cellular problems they may cause.[30] The cytotoxicity of these materials might be enhanced when they are subjected to bleaching agents and contact the saliva. The saliva was reported to be responsible for the biodegradation of resin-based materials.[31] For this purpose, the present study was conducted to assess the cytotoxicity of different restorative materials subjected to both a 10% hydrogen peroxide bleaching agent and artificial saliva. Cell viability was detected by investigating the mitochondrial activity (the MTT assay) after 5, 10 and 15 days of bleaching agent application. Although the manufacturers of bleaching agents recommend the use of the products for 5–10 days, in previous studies evaluating the clinical outcomes of bleaching, the agent was applied for up to 15 days.[29,32,33] Therefore, in the present study, 15 days of usage was also simulated, considering the possible over-treatment.

All the evaluated restorative materials were also immersed in PBS to assess and compare the biocompatibility of the materials without bleaching agent application. Besides, PBS was aimed to serve as a control to artificial saliva. The RNC specimens showed significantly the highest cell viability among the tested materials for all periods, followed by LDC and NHC (Table 3, Fig. 3). This finding is in accordance with the results of a recent study, which reported that the same RNC material exhibited higher HEK293 epithelial cell viability than LDC at the end of the 7th day.[34]

Although ceramics are known as inert and biocompatible materials with no cytotoxic effects,[35] the suppressed cellular activity caused by LDC was reported in previous studies.[36,37] This cytotoxic response has been related to mass release from the material. The presence of zinc (Zn) in LDC may influence cytotoxicity, since this element is considered a cellular-viability suppressor.[38] Previous studies also reported that the cytotoxicity of LDC decreased with time,[36,37,39] which was possibly due to the fact that the surface of the material adapted to the organic environment at the end of a two-weeks period.[36] Distinctively, in the present study, cell viability did not decrease in the LDC group immersed in PBS at the end of the test period, but it was also the case in the RNC group. However, the bleaching procedure caused a significant decrease in cell viability, possibly due to the alteration which occurred on the LDC surface treated with the bleaching agent.

Ceramics may leach and etch simultaneously when exposed to the saliva, and different ions may be released,[40] which results in biological responses, depending on the element type. Resin-based materials are also affected by contact with the saliva in terms of monomer release, since the saliva enhances the decomposition of monomers from the surface of the material. The exposure of resin-based materials to artificial saliva for 2 weeks demonstrated a further increase in the release of the decomposed monomers, and thereby increased the cytotoxic effect on epithelial cells.[41] Therefore, the restorative materials tested in this study were subjected to artificial saliva for the assessment of the influence of the saliva on cell viability with or without bleaching agent application. For all the evaluated restorative materials, significant differences were found in cell viability between the 5th and 15th day in the artificial saliva-only groups, and these viability values were significantly lower as compared to the control group. These results may be attributed to the release of elements or monomers from the restorative materials, which could be cytotoxic to epithelial cells. The artificial saliva used in the present study included sodium azide (NaN_3), which has been shown to reduce cell viability in high concentrations.[42] Despite the fact that a non-toxic concentration was used in this study (1 mM), the cytotoxic behavior of artificial saliva may be attributed to the content of this compound.

Although a lot of research has been conducted regarding the cytotoxicity of restorative materials, a limited number of those studies referred to the impact of bleaching agents in this respect. It is recommended to replace the existing restorations or re-polish their surfaces after bleaching to prevent discoloration or plaque accumulation, which may occur due to the surface alterations caused by the bleaching procedure.[43] Since over-the-counter bleaching products are not applied under the supervision of the clinician, their effects on the physical, optical and biological properties of restorative materials are of great concern. Therefore, this type of bleaching agents was preferred in the present study.

The specimens exposed to hydrogen peroxide for 30 min were immersed in either PBS or artificial saliva for the rest of the day after removing the bleaching agent from their surfaces. Regardless of the storage medium, all the bleached restorative materials significantly decreased cell viability as compared to the control group. However, when the cytotoxicity of the bleached and non-bleached materials immersed in artificial saliva was evaluated, significantly lower cell viability was detected in the LDC group, which indicates that the cytotoxicity behavior of RNC and NHC did not depend on the application of the bleaching agent. The lowest cell viability observed in the NHC groups can be related to the release of monomers from the material, induced by bleaching agent application and/or immersion in artificial saliva. The NHC material tested in the present study is composed of some monomers, of which BisGMA, UDMA[25] and TEGDMA[25,44] have been reported to have cytotoxic effects on certain cell types.[25] Volk et al. indicated that TEGDMA in combination with hydrogen peroxide significantly decreased the viability of human oral cells, even in low concentrations.[45] Therefore, the significant decrease in the viability of cells exposed to the bleached NHC may be attributed to the cytotoxic effects of the monomers released from the material.

The RNC material exhibited biocompatible behavior, with higher epithelial cell viability values, which is in accordance with recent studies.[34,46] Although RNC includes monomers such as BisGMA, UDMA, BisEMA, and TEGDMA, similar to NHC, the highly cross-linked polymer content of the material is 20%, which is lower than in the case of NHC, and RNC includes Zr particles. Moreover, the fabrication of RNC blocks is carried out under well-controlled temperatures and pressures, which enhances the final polymerization through eliminating shrinkage[45] and ensures that the UDMA monomer is bonded to the ceramic network with high strength.[47] Thus, although both RNC and NHC have resin content, the materials display different cytotoxicity behavior, which can be explained by differences in the microstructures and manufacturing processes of the materials.

Limitations

In the present study, the surfaces of the specimens were polished to obtain a standardized surface and to eliminate any irregularities. However, invisible porosities or cracks might exist or may have occurred due to bleaching agent application, and these irregularities could cause the storage of hydrogen peroxide, even after the cleaning procedure, which might have affected cell viability. Nevertheless, it should be taken into consideration that such surface irregularities can also be found on the surfaces of restorations.

In the present study, to better simulate the oral conditions, the assays were conducted using extracts from the culture medium and not by direct contact. A colorimetric MTT assay was performed to evaluate the viability of human embryonic kidney epithelial cells. Since the cytotoxicity was evaluated with the use of only the MTT assay, this may be regarded as a limitation, and other cytotoxicity tests involving gingival epithelial cells should be carried out for a better evaluation in future studies.

The determination of the release of monomers or elements, as well as scanning the surfaces of the materials after applying the test protocols can reflect the effect of the bleaching procedures on the materials in a more interpretive way, and can be the subject of further investigations.

Another limitation was the surface finishing of the LDC material, as LDC surfaces are glazed before clinical use; this may have affected the behavior of LDC. However, all the materials were subjected only to polishing in order to supply a standard protocol.

Conclusions

Within the limitations of the study, it can be concluded that over-the-counter home bleaching agents decrease cell viability when in contact with the LDC material. Cell viability was time-dependent, and significantly decreased at the end of the 15th day for all the bleached and non-bleached materials. Storage media and bleaching agents may affect the cytotoxicity behavior of restorative materials. Patients should be informed about these potential biological responses to over-the-counter whitening products and shorter periods of use should be recommended to minimize the cytotoxic effects.

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

Cenk Serhan Ozverel https://orcid.org/0000-0001-9932-4774
Sevcan Kurtulmus-Yilmaz https://orcid.org/0000-0001-8792-1977

References

1. Blatz MB, Conejo J. The current state of chairside digital dentistry and materials. *Dent Clin North Am*. 2019;63(2):175–197. doi:10.1016/j.cden.2018.11.002
2. Amesti-Garaizabal A, Agustín-Panadero R, Verdejo-Solá B, et al. Fracture resistance of partial indirect restorations made with CAD/CAM technology. A systematic review and meta-analysis. *J Clin Med*. 2019;8(11):1932. doi:10.3390/jcm8111932

3. Awada A, Nathanson D. Mechanical properties of resin-ceramic CAD/CAM restorative materials. *J Prosthet Dent*. 2015;114(4):587–593. doi:10.1016/j.prosdent.2015.04.016
4. Kurtulmus-Yilmaz S, Cengiz E, Ongun S, Karakaya I. The effect of surface treatments on the mechanical and optical behaviors of CAD/CAM restorative materials. *J Prosthodont*. 2019;28(2):e496–e503. doi:10.1111/jopr.12749
5. Goujat A, Abouelleil H, Colon P, et al. Mechanical properties and internal fit of 4 CAD-CAM block materials. *J Prosthet Dent*. 2018;119(3):384–389. doi:10.1016/j.prosdent.2017.03.001
6. Lawson NC, Bansal R, Burgess JO. Wear, strength, modulus and hardness of CAD/CAM restorative materials. *Dent Mater*. 2016;32(11):e275–e283. doi:10.1016/j.dental.2016.08.222
7. Stawarczyk B, Liebermann A, Eichberger M, Güth JF. Evaluation of mechanical and optical behavior of current esthetic dental restorative CAD/CAM composites. *J Mech Behav Biomed Mater*. 2015;55:1–11. doi:10.1016/j.jmbbm.2015.10.004
8. Nguyen JF, Migonney V, Ruse ND, Sadoun M. Properties of experimental urethane dimethacrylate-based dental resin composite blocks obtained via thermo-polymerization under high pressure. *Dent Mater*. 2013;29(5):535–541. doi:10.1016/j.dental.2013.02.006
9. Lauvahutanon S, Takahashi H, Shiozawa M, et al. Mechanical properties of composite resin blocks for CAD/CAM. *Dent Mater J*. 2014;33(5):705–710. doi:10.4012/dmj.2014-208
10. Hussain B, Le Thieu MK, Johnsen GF, Reseland JE, Haugen HJ. Can CAD/CAM resin blocks be considered as substitute for conventional resins? *Dent Mater*. 2017;33(12):1362–1370. doi:10.1016/j.dental.2017.09.003
11. Kwon SR, Wertz PW. Review of the mechanism of tooth whitening. *J Esthet Restor Dent*. 2015;27(5):240–257. doi:10.1111/jerd.12152
12. Haywood VB. History, safety, and effectiveness of current bleaching techniques and applications of the nightguard vital bleaching technique. *Quintessence Int*. 1992;23(7):471–488. PMID:1410249.
13. Minoux M, Serfaty R. Vital tooth bleaching: Biologic adverse effects – a review. *Quintessence Int*. 2008;39(8):645–659. PMID:19107251.
14. Celik C, Yüzügüllü B, Erkut S, Yazici AR. Effect of bleaching on staining susceptibility of resin composite restorative materials. *J Esthet Restor Dent*. 2009;21(6):407–414. doi:10.1111/j.1708-8240.2009.00299.x
15. Kurtulmus-Yilmaz S, Cengiz E, Ulusoy N, Ozak ST, Yuksel E. The effect of home-bleaching application on the color and translucency of five resin composites. *J Dent*. 2013;41(Suppl 5):e70–e75. doi:10.1016/j.jdent.2012.12.007
16. Cengiz E, Kurtulmus-Yilmaz S, Ulusoy N, Deniz ST, Yuksel-Devrim E. The effect of home bleaching agents on the surface roughness of five different composite resins: A SEM evaluation. *Scanning*. 2016;38(3):277–283. doi:10.1002/sca.21307
17. Bezerra Rattacaso RM, da Fonseca Roberti Garcia L, Aguilar FG, Consani S, de Carvalho Panzeri Pires-de-Souza F. Bleaching agent action on color stability, surface roughness and microhardness of composites submitted to accelerated artificial aging. *Eur J Dent*. 2011;5(2):143–149. PMID:21494380. PMCID:PMC3075998.
18. Rea FT, Cabral Roque AC, Macedo AP, de Almeida RP. Effect of carbamide peroxide bleaching agent on the surface roughness and gloss of a pressable ceramic. *J Esthet Restor Dent*. 2019;31(5):451–456. doi:10.1111/jerd.12469
19. Demir N, Karci M, Ozcan M. Effects of 16% carbamide peroxide bleaching on the surface properties of glazed glassy matrix ceramics. *Biomed Res Int*. 2020;2020:1864298. doi:10.1155/2020/1864298
20. Yu H, Zhang CY, Wang YN, Cheng H. Hydrogen peroxide bleaching induces changes in the physical properties of dental restorative materials: Effects of study protocols. *J Esthet Restor Dent*. 2018;30(2):E52–E60. doi:10.1111/jerd.12345
21. Karakaya İ, Cengiz E. Effect of 2 bleaching agents with a content of high concentrated hydrogen peroxide on stained 2 CAD/CAM blocks and a nanohybrid composite resin: An AFM evaluation. *Biomed Res Int*. 2017;2017:6347145. doi:10.1155/2017/6347145
22. Abd Elhamid M, Mosallam R. Effect of bleaching versus repolishing on colour and surface topography of stained resin composite. *Aust Dent J*. 2010;55(4):390–398. doi:10.1111/j.1834-7819.2010.01259.x
23. Tabatabaee MH, Arami S, Ghavam M, Rezaii A. Monomer release from nanofilled and microhybrid dental composites after bleaching. *J Dent (Tehran)*. 2014;11(1):56–66. PMID:24910677. PMCID:PMC4037267.
24. Gul P, Karatas O, Alp HH, Cam IB, Ozakar-Ilday N. Monomer release from nanohybrid composites after bleaching. *J Oral Sci*. 2019;61(2):351–357. doi:10.2334/josnusd.18-0063
25. Yoshii E. Cytotoxic effects of acrylates and methacrylates: Relationships of monomer structures and cytotoxicity. *J Biomed Mater Res*. 1997;37(4):517–524. doi:10.1002/(sici)1097-4636(19971215)37:4<517::aid-jbm10>3.0.co;2-5
26. Rodríguez-Martínez J, Valiente M, Sánchez-Martín MJ. Tooth whitening: From the established treatments to novel approaches to prevent side effects. *J Esthet Restor Dent*. 2019;31(5):431–440. doi:10.1111/jerd.12519
27. Haywood VB. Nightguard vital bleaching: Construction of NGVB prosthetic. *Dent Today*. 1997;16(6):86–91.
28. Boushell LW, Ritter AV, Garland GE, et al. Nightguard vital bleaching: Side effects and patient satisfaction 10 to 17 years post-treatment. *J Esthet Restor Dent*. 2012;24(3):211–219. doi:10.1111/j.1708-8240.2011.00479.x
29. Carlos NR, Bridi EC, Amaral F, França F, Turssi CP, Basting RT. Efficacy of home-use bleaching agents delivered in customized or prefilled disposable trays: A randomized clinical trial. *Oper Dent*. 2017;42(1):30–40. doi:10.2341/15-315-C
30. Wataha JC, Hanks CT, Strawn SE, Fat JC. Cytotoxicity of components of resins and other dental restorative materials. *J Oral Rehabil*. 1994;21(4):453–462. doi:10.1111/j.1365-2842.1994.tb01159.x
31. Lin BA, Jaffer F, Duff MD, Tang YW, Santerre JP. Identifying enzyme activities within human saliva which are relevant to dental resin composite biodegradation. *Biomaterials*. 2005;26(20):4259–4264. doi:10.1016/j.biomaterials.2004.11.001
32. Cordeiro D, Toda C, Hanan S, et al. Clinical evaluation of different delivery methods of at-home bleaching gels composed of 10% hydrogen peroxide. *Oper Dent*. 2019;44(1):13–23. doi:10.2341/17-174-C
33. Dourado Pinto AV, Carlos NR, Botelho do Amaral FL, Gomes França FM, Turssi CP, Basting RT. At-home, in-office and combined dental bleaching techniques using hydrogen peroxide: Randomized clinical trial evaluation of effectiveness, clinical parameters and enamel mineral content. *Am J Dent*. 2019;32(3):124–132. PMID:31295393.
34. Atay A, Gürdal I, Çetıntas VB, Üşümez A, Cal E. Effects of new generation all-ceramic and provisional materials on fibroblast cells. *J Prosthodont*. 2019;28(1):e383–e394. doi:10.1111/jopr.12915
35. Leinfelder KF. Ask the expert. Will ceramic restorations be challenged in the future? *J Am Dent Assoc*. 2001;132(1):46–47. doi:10.14219/jada.archive.2001.0024
36. Messer RL, Lockwood PE, Wataha JC, Lewis JB, Norris S, Boillaguet S. In vitro cytotoxicity of traditional versus contemporary dental ceramics. *J Prosthet Dent*. 2003;90(5):452–458. doi:10.1016/s0022-3913(03)00533-x
37. Brackett MG, Lockwood PE, Messer RL, Lewis JB, Boillaguet S, Wataha JC. In vitro cytotoxic response to lithium disilicate dental ceramics. *Dent Mater*. 2008;24(4):450–456. doi:10.1016/j.dental.2007.06.013
38. Wataha JC, Hanks CT, Craig RG. The in vitro effects of metal cations on eukaryotic cell metabolism. *J Biomed Mater Res*. 1991;25(9):1133–1149. doi:10.1002/jbm.820250907
39. Rizo-Gorrita M, Herráez-Galindo C, Torres-Lagares D, Serrera-Figallo MÁ, Gutiérre-Pérez JL. Biocompatibility of polymer and ceramic CAD/CAM materials with human gingival fibroblasts (HGFs). *Polymers (Basel)*. 2019;11(9):1446. doi:10.3390/polym11091446
40. Dündar M, Artunç C, Toksavul S, Ozmen D, Turgan N. Determination of elemental composition of substance lost following wear of all-ceramic materials. *Int J Prosthodont*. 2003;16(3):261–264. PMID:12854789.
41. Wataha JC, Rueggeberg FA, Lapp CA, et al. In vitro cytotoxicity of resin-containing restorative materials after aging in artificial saliva. *Clin Oral Investig*. 1999;3(3):144–149. doi:10.1007/s007840050093
42. Ji D, Kamalden TA, del Olmo-Aguado S, Osborne NN. Light- and sodium azide-induced death of RGC-5 cells in culture occurs via different mechanisms. *Apoptosis*. 2011;16(4):425–437. doi:10.1007/s10495-011-0574-4
43. Rodrigues CS, Nora BD, Mallmann A, May LG, Jacques LB. Repolishing resin composites after bleaching treatments: Effects on color stability and smoothness. *Oper Dent*. 2019;44(1):54–64. doi:10.2341/17-107-L

44. Stanislawski L, Lefeuvre M, Bourd K, Soheili-Majd E, Goldberg M, Périanin A. TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. *J Biomed Mater Res A*. 2003;66(3):476–482. doi:10.1002/jbm.a.10600
45. Volk J, Leyhausen G, Dogan S, Geurtsen W. Additive effects of TEGDMA and hydrogen peroxide on the cellular glutathione content of human gingival fibroblasts. *Dent Mater.* 2007;23(8):921–926. doi:10.1016/j.dental.2006.08.001
46. Campaner M, Takamiya AS, Bitencourt SB, et al. Cytotoxicity and inflammatory response of different types of provisional restorative materials. *Arch Oral Biol*. 2020;111:104643. doi:10.1016/j.archoralbio.2019.104643
47. Nguyen JF, Ruse D, Phan AC, Sadoun MJ. High-temperature-pressure polymerized resin-infiltrated ceramic networks. *J Dent Res*. 2014;93(1):62–67. doi:10.1177/0022034513511972

Original paper

Mechanism of enamel damage in the grooves of molars during mastication

Beata Dejak[1,A–F], Elżbieta Bołtacz-Rzepkowska[2,D,F]

[1] Department of Prosthetic Dentistry, Medical University of Lodz, Poland
[2] Department of Conservative Dentistry, Medical University of 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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):321–326

Address for correspondence
Beata Dejak
E-mail: beata.dejak@umed.lodz.pl

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on January 16, 2022
Reviewed on September 9, 2022
Accepted on September 19, 2022

Published online on February 20, 2023

Abstract

Background. During mastication, molars are subjected to heavy stress. However, a full explanation of the effects of physiological loads on tooth structures is lacking.

Objectives. The study aimed to determine stress in molars and identify the mechanism of enamel damage in the grooves of the teeth during computer-simulated mastication.

Material and methods. The study was carried out using the finite element method (FEM). A three-dimensional (3D) model of the first mandibular molar and of the crown of the opposing maxillary tooth was created. A food bite was introduced between the antagonistic teeth. The mastication cycle of the bolus was computer-simulated. The equivalent stress in the enamel and dentin of the mandibular molar was calculated according to the modified von Mises (mvM) criterion.

Results. During the simulated chewing activity, the highest equivalent mvM stress and tensile stress concentrated on the molar enamel around the central groove and the foramen cecum. The value of the equivalent mvM stress was close to the tensile strength of the enamel. According to the mvM criterion, the enamel in these areas was exposed to destruction, which coincided with the occurrence of class I caries.

Conclusions. During mastication, significant tensile and mvM stress concentrates on the mandibular molar enamel around the central groove and the foramen cecum. High stress in these areas may cause prism microfractures and facilitate the bacterial penetration of the enamel.

Keywords: finite element analysis, modified von Mises failure criterion, enamel damage, biomechanical causes of caries, simulation of mastication

Cite as
Dejak B, Bołtacz-Rzepkowska E. Mechanism of enamel damage in the grooves of molars during mastication. *Dent Med Probl.* 2023;60(2):321–326. doi:10.17219/dmp/154777

DOI
10.17219/dmp/154777

Introduction

The enamel is the hardest, mineralized, cell-free form of tissue in the human body. Hydroxyapatite (HA) – calcium fluoridated carbonated apatite crystals – constitutes 96% of the enamel. It is organized in 20-nanometer nanospheres.[1] They form HA crystal nanoparticles. These nanoparticles are long, ribbon-like structures with a hexagonal cross-section, and are approx. 50–70 nm wide, 20–25 nm thick and more than twice as long.[1] The crystals are joined by glycoproteins (2 nm wide) to form enamel prisms, which are mostly arranged perpendicular to the dentin–enamel junction (DEJ). The tissue thickness ranges from 0.01 mm in the cervical region to 2–2.5 mm at the peaks of the molar cusps.[2] The occlusal surfaces of the teeth are anatomically diverse and the cusps are separated by a system of grooves. The depth of the central groove depends on its shape (it can be I-shaped, U-shaped, V-shaped, or inverted Y (IY)-shaped) and ranges from 0.53 mm to 1.15 mm.[3]

The enamel has unique mechanical properties. It is rigid, with an elastic modulus of 84.1 GPa, though the value varies (70 GPa at DEJ and 115 GPa near the occlusal surfaces).[4,5] It is also very hard and has a Vickers microhardness of 4 GPa, which is lower at DEJ (3 GPa) and higher near the occlusal surfaces (6 GPa).[6] Meanwhile, its compressive strength is high (363.0 MPa)[7] and its tensile strength is very low (10.0–11.4 MPa) (Table 1).[8] The stiffness, hardness and compressive strength of the enamel make it resistant to mechanical stimuli within the oral cavity. However, its highly mineralized composition means it is a glass-like biomaterial, prone to breakage (brittleness: 300–900 μm^{-1}; fracture toughness (Kc): 0.67–0.95 $MPa \cdot m^{1/2}$).[9,10] The effects of the chewing forces on tooth tissues, as well as stress distribution in the enamel and dentin under occlusal loads, are yet to be fully understood.

The effects of the forces acting on the teeth can be investigated using finite element analysis (FEA). Research in this area has mainly concerned different restorative materials and various shapes of fillings or prosthetic appliances, and has rarely focused on healthy teeth.[11,12] Also, most authors study the von Mises or principal stress in the teeth.[11–13] Here, the modified von Mises (mvM) criterion was used to consider the difference between the compressive and tensile strength of the enamel and dentin. The calculated mvM stress values reflected the actual stress in tooth tissues.

The load used in modeling massively impacts stress distribution. In most finite element method (FEM) studies, static forces are applied directly to the occlusal surfaces.[11,12] However, the current study used a computer model based on the natural interarch relationship. Dynamic three-dimensional (3D) simulations of the bolus mastication cycle were performed using contact elements on the occlusal surfaces, and the molar tooth was loaded with variable chewing forces transmitted by the food bolus to the enamel. Due to this innovative approach, stress in molar structures was investigated under realistic conditions.

The present study aimed to determine stress in molars and identify the mechanism of enamel damage in the grooves of the teeth during computer-simulated mastication.

Material and methods

Creating tooth models for finite element analysis

The impressions of the maxillary and mandibular teeth were taken from a patient with normal occlusion, using the Express™ polyvinyl siloxane material (3M Espe Dental Products, St. Paul, USA). Based on the impressions, plaster models were cast using class IV Giroform® stone (Amann Girrbach GmbH, Pforzheim, Germany). The scans of the plaster models of the right mandibular first molar and of the opposing maxillary first molar were made using the Ceramill MAP 300 scanner (Amann Girrbach AG, Kolbach, Austria), and then they were processed using the Ceramill Mind software (Amann Girrbach AG). The presentation timestamp (PTS) files containing the coordinates of the tooth surface points were loaded into the Ansys computer program, v. 14 (Ansys, Inc., Canonsburg, USA) for FEA.[14] In the same patient, cone-beam computed tomography (CBCT) of the right mandibular first molar tooth was performed by means of the CS 9300 system (Carestream Dental, Atlanta, USA). The CT scans were used to obtain the points along DEJ and within the chamber of the molar. The selected points were then connected with curves in frontal planes every 0.1 mm, using a preprocessor. Based on these lines, a solid model of the intact molar was created, taking into account the enamel, dentin and tooth chamber. The shape and dimensions of the tooth model corresponded to an average first molar.[15] The scan of the surface of the right maxillary first molar made it possible to generate a model of a fragment of the tooth crown (Fig. 1).

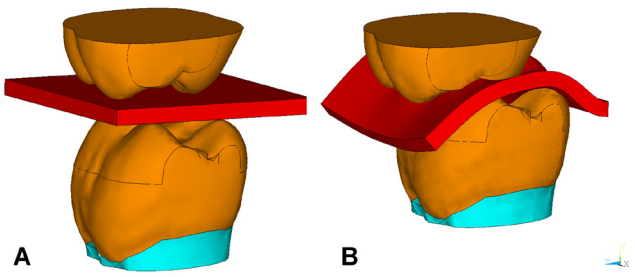

Fig. 1. Models of molars during the simulation of mastication

A – initial closing phase of the mastication cycle (the right mandibular first molar and a fragment of the crown of the opposing tooth in a lateral position, with a morsel between the teeth); B – final closing phase of the mastication cycle (the right mandibular first molar and a fragment of the crown of the opposing tooth in central occlusion, with a morsel between the teeth).

Table 1. Properties of the materials used in the models

Material	Modulus of elasticity	Poisson's ratio	Ultimate compressive strength [MPa]	Ultimate tensile strength [MPa]
Enamel	84.1 GPa	0.33	363.0	10.0–11.4
Dentin	18.6 GPa	0.31	297.0	99.8
Food bolus	21.6 MPa	0.30	–	–

The maxillary crown model was fixed on its upper surface in the nodes, and the opposing tooth model was set in lateral occlusion and vertically spaced apart by 1 mm. A 1-millimeter-thick morsel of food was then inserted between the opposing teeth, creating a 3D computer model of the opposing molars in the initial closing phase of the mastication cycle (Fig. 1A).

Model materials

The values for the modulus of elasticity and Poisson's ratio were entered for the enamel[4] and dentin.[16] The food bolus had the properties of a nut, and its elastic modulus was 21.6 MPa.[17] The tensile strength values were added for the enamel (10.0–11.4 MPa)[8] and dentin (99.8 MPa),[18] as well as the compressive strength values (363.0 MPa[7] and 297.0 MPa,[19] respectively). The materials used in the models were elastic, homogeneous and isotropic, but had different compressive and tensile strength (Table 1).

Dividing the models into finite elements

For calculations, each tooth model was divided into 10-node structural elements (Solid 187). In total, 61,801 elements joined by 84,215 nodes were used. Pairs of contact elements, Targe 170 and Conta 174, were used on the occlusal surfaces of the teeth and the bolus. The coefficient of friction on the contact surfaces was assumed to be 0.2.[20]

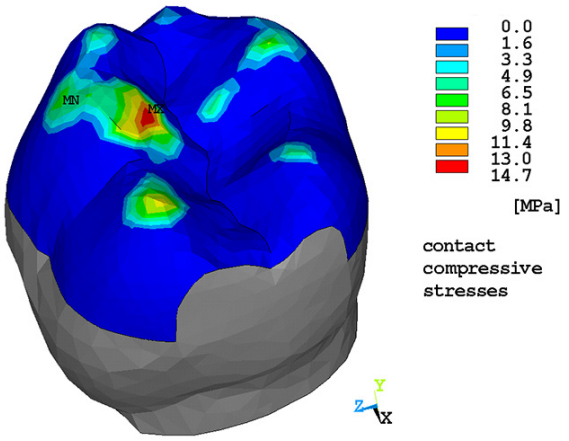

Fig. 2. Pressure exerted on the occlusal surface of the mandibular molar at the end of the closing phase of the mastication cycle

MN – minimum; MX – maximum.

Model loading

A computer simulation of the closing phase of the mastication cycle was performed. The mandibular molar was moved vertically upward, and simultaneously medially and mesially toward the maxillary tooth until maximum intercuspation was achieved. The nodes on the lower surface of the mandibular crown were then displaced, with the vertical displacements chosen so that the maximum reaction force toward the Y-axis in each model was 100 N.[21] The buccal cusps of the lower tooth glided down the bolus and along the occlusal surface of the upper tooth, thereby crushing the bolus (Fig. 1B).[22] In this way, the natural load on the molar during mastication was computer-simulated.

Calculations

The contact simulation performed with the use of FEM is a non-linear analysis. During the masticatory simulation, the pressure exerted on the occlusal surface of the mandibular first molar was investigated and the stress components in the tooth were calculated. The enamel and dentin have different compressive and tensile strength. Therefore, the mvM criterion was used to evaluate the enamel and dentine strain in complex stress states.[23] According to this criterion, the material will fail when the value of the equivalent mvM stress exceeds the tensile strength of the material.

Results

The highest, unevenly distributed pressure was exerted on the occlusal surface of the mandibular molar by the crushed bolus during the final closing phase of the mastication cycle. A maximum pressure of 14.7 MPa was exerted on the tops and slopes of the working cusps (Fig. 2). The buccal and lingual cusps were pushed apart during loading, and the equivalent mvM stress reached around the central groove in the enamel of the intact tooth was 9.75 MPa (Fig. 3), which is very close to the tensile strength of the enamel.[8] Meanwhile, the tensile stress in the grooves was 6.34 MPa (Fig. 4). The equivalent mvM stress of 4.86 MPa occurred around the foramen cecum (Fig. 3). In the dentin, a maximum mvM stress of 21 MPa concentrated at the tooth cervix on the buccal side (Fig. 5). However, this stress value was 5 times lower than the tensile strength of the dentine (99.8 MPa).[18]

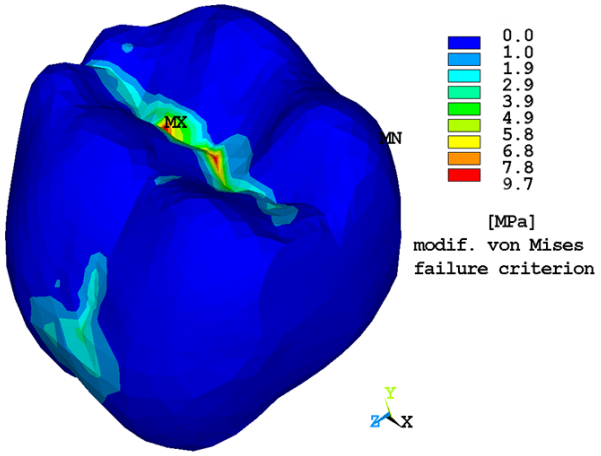

Fig. 3. Distribution and values of the equivalent stress according to the modified von Mises (mvM) criterion in the enamel of the mandibular molar at the end of the closing phase of the mastication cycle

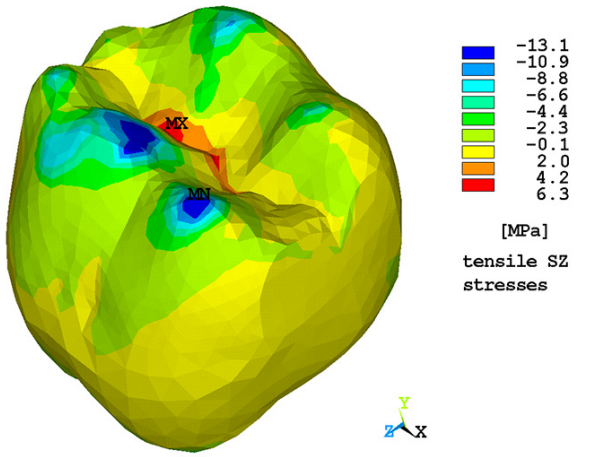

Fig. 4. Distribution and values of the tensile SZ (in the B–L direction) stress in the mandibular molar at the end of the closing phase of the mastication cycle

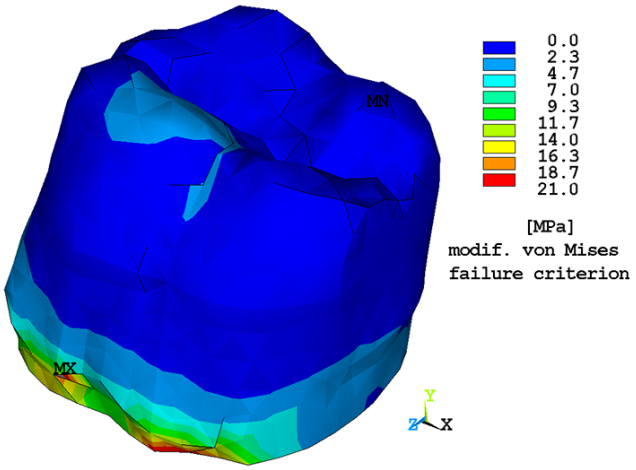

Fig. 5. Distribution and values of the equivalent stress according to the modified von Mises (mvM) criterion in the dentin of the mandibular molar at the end of the closing phase of the mastication cycle

Discussion

The present study demonstrated that the cusps of the molar are subjected to bending and are pushed away from each other during normal mastication, which confirms the findings of other experimental studies.[24] In the enamel, the highest tensile stress and the greatest strain occurred around the central groove of the tooth. Meanwhile, the highest value of the equivalent mvM stress was also observed around the central groove of the molar, and it was close to the tensile strength of the enamel. The enamel has different compressive and tensile strength, and is characterized by a low tensile strength (10.0–11.4 MPa) due to the perpendicularly oriented enamel prisms.[8] According to the mvM criterion, the enamel in this area was exposed to destruction. Indeed, the enamel in the tooth grooves may fail when it is subjected to cyclic forces greater than 100 N. The maximum bite force in the molar region in dentate patients varies between 402.07 N and 686.46 N, and for natural mastication, it varies between 68.64 N and 147.10 N.[25]

The FEM studies by Benazzi et al.[13,26] and Magne and Belser[27] confirm our results. Magne and Belser demonstrated that the pressure exerted on the non-working cusps was particularly dangerous and caused high tensile stress in the teeth.[27] Benazzi et al. found that the greatest tensile stress occurred in the grooves during maximum intercuspation.[26] According to Wan et al., the horizontal component of the masticatory forces opens the spaces between the central grooves.[28] Particularly sharp angles and narrow curves within the fissure system generate concentrated stress. At the bottom of the fissures, especially the I-shaped, V-shaped and IY-shaped ones, enamel cracks initiate and propagate into the enamel to a depth of 1.04–1.25 mm.[28]

The mechanism of enamel failure during loading was presented by Xia et al.[1] Enamel crystals respond to the mastication forces at nanoscales in 3 distinct ways: plucking; plastic deformation; and fragmentation. The plucking of HA nanoparticles occurs when the forces exceed the strength of the protein. In particular, the tensile stress acting perpendicularly to the prisms is dangerous.[1] The present study showed that mastication contributed to an unfavorable distribution of tensile stress in the central groove of the enamel, which can predispose to collagen breakdown and the microfractures of the enamel prisms. The continuous repetition of the process can reduce the integrity of the enamel. To date, it has not been demonstrated that physiological loads on molars contribute to the creation of significant tensile stress in the anatomical cavities of the enamel, which may lead to the microfractures of the enamel prisms in these places.

According to Ricucci et al., bacterial biofilms colonize cracks in the enamel consistently.[29] Moreover, it was demonstrated by Walker et al. that the cracked enamel was permeable to dyes and cariogenic bacteria.[10] As such,

cracks open the way for bacterial invasion and are one of the causes of tooth decay. Furthermore, cyclic mechanical loads increase the penetration of bacteria into narrow tooth gaps.[30,31] In the absence of occlusive pressure, the degree of bacterial penetration into the dental gaps is approx. 30 μm (67%). However, this increases to 100% during the cyclic loading of the tooth.[30] The current study showed the perfect convergence of the location of the maximum equivalent mvM stress in the enamel generated during mastication with the occurrence of class I caries in the fissures and the foramen cecum.

Dental caries is a major oral disease and the most common dental disorder of multifactorial etiology. The modern concept of caries etiology describes an imbalance between the microbial load and lifestyle, the protective role of saliva, and the enamel resistance. The development of caries is accompanied by key components, such as bacterial plaque, carbohydrates and dental susceptibility.[32] The main mass of the biofilm adhering to the surfaces of the teeth consists of spatially organized bacteria surrounded by an extracellular matrix. The consumption of carbohydrates increases the number of carious bacteria and the cariogenicity of the plaque through acidic fermentation products.[33] The carious process begins with changes in the enamel, and the susceptibility of the enamel depends on the degree of hard tissue mineralization and the anatomical structure of the tooth.[34] Bacteria do not colonize all tooth surfaces equally, and caries is most common on the occlusal surfaces of molar teeth.[35] Indeed, narrow fissures on the occlusal surface, such as grooves and anatomical depressions, are particularly prone to biofilm retention.[36] Bacterial colonies were noted in the prismatic structures of the enamel and the interprismatic substance, even within an intact groove-fossa system.[37] Unfortunately, these areas are resistant to natural abrasion, hygiene methods and the protective properties of saliva.

This paper presents the mechanism of enamel destruction in the molar grooves as a result of masticatory mechanical loads. Furthermore, areas in the molar enamel where significant tensile stress occurred corresponded to areas that were affected by class I caries. Two mechanisms may contribute to the formation of class I caries in molars, i.e., the destruction of the enamel prisms in the grooves due to tensile stress that exceeds the enamel strength, and the penetration of bacteria deep into the grooves during cyclic loading. Mechanical damage to the enamel prisms can act as a gateway to bacterial infection and biofilm retention, and tooth biomechanics can be considered one of the factors that contribute to the initiation of dental caries.

Research and treatment in modern dentistry are increasingly based on numerical methods, as they are more accurate and provide more possibilities than the conventional methods. Indeed, computerized kinematic facebows are preferred over mechanical facebows,[38] intraoral scanners have an advantage over traditional impressions,[39] the computer-aided design (CAD) and computer-aided manufacturing (CAM) of prosthetic restorations are now commonly used, and models can be 3D-printed instead of being plaster-cast.[40] The use of FEM in this study made it possible to visualize the distribution of stress in tooth tissues during the simulation of mastication.

Conclusions

Taking into account the limitations of the method, the following conclusions can be drawn:

– during mastication, significant tensile and mvM stress concentrates on the mandibular molar enamel around the central groove and the foramen cecum;
– high stress in these areas may cause prism microfractures and facilitate the bacterial penetration of the enamel; it coincides with the occurrence of class I caries.

Ethics approval and consent to participate

The study was approved by the Bioethics Committee at the Medical University of Lodz, Poland (approval No. RNN/98/09/KE UM). The informed written consent was obtained from the patient.

Data availability

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

Consent for publication

Not applicable.

ORCID iDs

Beata Dejak https://orcid.org/0000-0001-7469-5691
Elżbieta Bołtacz-Rzepkowska https://orcid.org/0000-0002-1696-0618

References

1. Xia J, Tian ZR, Hua L, et al. Enamel crystallite strength and wear: Nanoscale responses of teeth to chewing loads. *J R Soc Interface*. 2017;14(135):20170456. doi:10.1098/rsif.2017.0456
2. Nanci A, ed. *Ten Cate's Oral Histology. Development, Structure, and Function*. 9th ed. St. Louis, MO: Elsevier; 2018:289.
3. Khanna R, Pandey RK, Singh N. Morphology of pits and fissures reviewed through scanning electron microscope. *Dentistry*. 2015;5(4):100287. doi:10.4172/2161-1122.1000287
4. Habelitz S, Marshall SJ, Marshall GW Jr., Balooch M. Mechanical properties of human dental enamel on the nanometre scale. *Arch Oral Biol*. 2001;46(2):173–183. doi:10.1016/s0003-9969(00)00089-3
5. Cuy JL, Mann AB, Livi KJ, Teaford MF, Weihs TP. Nanoindentation mapping of the mechanical properties of human molar tooth enamel. *Arch Oral Biol*. 2002;47(4):281–291. doi:10.1016/s0003-9969(02)00006-7
6. Alamoush RA, Silikas N, Salim NA, Al-Nasrawi S, Satterthwaite JD. Effect of the composition of CAD/CAM composite blocks on mechanical properties. *Biomed Res Int*. 2018;2018:4893143. doi:10.1155/2018/4893143
7. Zaytsev D. Mechanical properties of human enamel under compression: On the feature of calculations. *Mater Sci Eng C Mater Biol Appl*. 2016;62:518–523. doi:10.1016/j.msec.2016.02.016

8. Giannini M, Soares CJ, de Carvalho RM. Ultimate tensile strength of tooth structures. *Dent Mater*. 2004;20(4):322–329. doi:10.1016/S0109-5641(03)00110-6
9. Park S, Quinn JB, Romberg E, Arola D. On the brittleness of enamel and selected dental materials. *Dent Mater*. 2008;24(11):1477–1485. doi:10.1016/j.dental.2008.03.007
10. Walker BN, Makinson OF, Peters MC. Enamel cracks. The role of enamel lamellae in caries initiation. *Aust Dent J*. 1998;43(2):110–116. doi:10.1111/j.1834-7819.1998.tb06099.x
11. Ausiello P, Ciaramella S, Fabianelli A, et al. Mechanical behavior of bulk direct composite versus block composite and lithium disilicate indirect Class II restorations by CAD – FEM modeling. *Dent Mater*. 2017;33(6):690–701. doi:10.1016/j.dental.2017.03.014
12. Magne P, Besler UC. Porcelain versus composite inlays/onlays: Effects of mechanical loads on stress distribution, adhesion, and crown flexure. *Int J Periodontics Restorative Dent*. 2003;23(6):543–555. PMID:14703758.
13. Benazzi S, Nguyen HN, Kullmer O, Kupczik K. Dynamic modelling of tooth deformation using occlusal kinematics and finite element analysis. *PLoS ONE*. 2016;11(3):e0152663. doi:10.1371/journal.pone.0152663
14. Zienkiewicz OC, Taylor RL. *The Finite Element Method. Volume 1: The Basis*. 5th ed. Oxford, UK: Butterworth-Heinemann; 2000:87–110.
15. Ash MM Jr., Nelson SJ. *Wheeler's Dental Anatomy, Physiology, and Occlusion*. 8th ed. St. Louis, MO: Saunders/Elsevier; 2003:297–306.
16. Ziskind D, Hasday M, Cohen SR, Wagner HD. Young's modulus of peritubular and intertubular human dentin by nano-indentation tests. *J Struct Biol*. 2011;174(1):23–30. doi:10.1016/j.jsb.2010.09.010
17. Agrawal KR, Lucas PW, Printz JF, Bruce IC. Mechanical properties of foods responsible for resisting food breakdown in the human mouth. *Arch Oral Biol*. 1997;42(1):1–9. doi:10.1016/s0003-9969(96)00102-1
18. Inoue S, Pereira PN, Kawamoto C, et al. Effect of depth and tubule direction on ultimate tensile strength of human coronal dentin. *Dent Mater J*. 2003;22(1):39–47. doi:10.4012/dmj.22.39
19. Craig RG, Powers JM, Wataha JC. *Dental Materials. Properties and Manipulation*. 11th ed. St. Louis, MO: Mosby; 2003:78.
20. Katona TR. A mathematical analysis of the role of friction in occlusal trauma. *J Prosthet Dent*. 2001;86(6):636–643. doi:10.1067/mpr.2001.120068
21. Gibbs CH, Mahan PE, Lundeen HC, Brehnan K, Walsh EK, Holbrook WB. Occlusal forces during chewing and swallowing as measured by sound transmission. *J Prost Dent*. 1981;46(4):443–449. doi:10.1016/0022-3913(81)90455-8
22. Rilo B, Fernández-Formoso N, Mora MJ, Cadarso-Suárez C, Santana U. Distance of the contact glide in the closing masticatory stroke during mastication of three types of food. *J Oral Rehabil*. 2009;36(8):571–576. doi:10.1111/j.1365-2842.2009.01956.x
23. De Groot R, Peters MC, De Haan YM, Dop GJ, Plasschaert AJ. Failure stress criteria for composite resin. *J Dent Res*. 1987;66(12):1748–1752. doi:10.1016/0022-3913(81)90455-8
24. Panitvisai P, Messer HH. Cuspal deflection in molars in relation to endodontic and restorative procedures. *J Endod*. 1995;21(2):57–61. doi:10.1016/s0099-2399(06)81095-2
25. Orchardson R, Cadden SW. Mastication and swallowing: 1. Functions, performance and mechanisms. *Dent Update*. 2009;36(6):327–330,332–334,337. doi:10.12968/denu.2009.36.6.327
26. Benazzi S, Kullmer O, Grosse IR, Weber GW. Using occlusal wear information and finite element analysis to investigate stress distributions in human molars. *J Anat*. 2011;219(3):259–272. doi:10.1111/j.1469-7580.2011.01396.x
27. Magne P, Belser UC. Rationalization of shape and related stress distribution in posterior teeth: A finite element study using nonlinear contact analysis. *Int J Periodontics Restorative Dent*. 2002;22(5):425–433. PMID:12449302.
28. Wan B, Shahmoradi M, Zhang Z, et al. Modelling of stress distribution and fracture in dental occlusal fissures. *Sci Rep*. 2019;9(1):4682. doi:10.1038/s41598-019-41304-z
29. Ricucci D, Siqueira JF Jr., Loghin S, Berman LH. The cracked tooth: Histopathologic and histobacteriologic aspects. *J Endod*. 2015;41(3):343–352. doi:10.1016/j.joen.2014.09.021
30. Khvostenko D, Salehi S, Naleway SE, et al. Cyclic mechanical loading promotes bacterial penetration along composite restoration marginal gaps. *Dent Mater*. 2015;31(6):702–710. doi:10.1016/j.dental.2015.03.011
31. Ferracane JL. Models of caries formation around dental composite restorations. *J Dent Res*. 2017;96(4):364–371. doi:10.1177/0022034516683395
32. Pitts NB, Zero DT, Marsh PD, et al. Dental caries. *Nat Rev Dis Primers*. 2017;3:17030. doi:10.1038/nrdp.2017.30
33. Carvalho JC, Dige I, Machiulskiene V, et al. Occlusal caries: Biological approach for its diagnosis and management. *Caries Res*. 2016;50(6):527–542. doi:10.1159/000448662
34. Mejàre I, Axelsson S, Dahlén G, et al. Caries risk assessment. A systematic review. *Acta Odontol Scand*. 2014;72(2):81–91. doi:10.3109/00016357.2013.822548
35. Hopcraft MS, Morgan MV. Pattern of dental caries experience on tooth surfaces in an adult population. *Community Dent Oral Epidemiol*. 2006;34(3):174–183. doi:10.1111/j.1600-0528.2006.00270.x
36. Carvalho JC. Caries process on occlusal surfaces: Evolving evidence and understanding. *Caries Res*. 2014;48(4):339–346. doi:10.1159/000356307
37. Ekstrand KR, Bjørndal L. Structural analyses of plaque and caries in relation to the morphology of the groove-fossa system on erupting mandibular third molars. *Caries Res*. 1997;31(5):336–348. doi:10.1159/000262416
38. Wieckiewicz M, Zietek M, Nowakowska D, Wieckiewicz W. Comparison of selected kinematic facebows applied to mandibular tracing. *Biomed Res Int*. 2014;2014:818694. doi:10.1155/2014/818694
39. Ren X, Son K, Lee KB. Accuracy of proximal and occlusal contacts of single implant crowns fabricated using different digital scan methods: An in vitro study. *Materials (Basel)*. 2021;14(11):2843. doi:10.3390/ma14112843
40. Raszewski Z, Kulbacka J, Nowakowska-Toporowska A. Mechanical properties, cytotoxicity, and fluoride ion release capacity of bioactive glass-modified methacrylate resin used in three-dimensional printing technology. *Materials (Basel)*. 2022;15(3):1133. doi:10.3390/ma15031133

Review

Effect of bonded and removable retainers on occlusal settling after orthodontic treatment: A systematic review and meta-analysis

Umair Shoukat Ali[1,A–E], Kamil Zafar[2,C,D], Rashna Hoshang Sukhia[3,E,F], Mubassar Fida[3,E,F], Aqeel Ahmed[3,B,D,E]

[1] Department of Orthodontics, Baqai Dental College, Karachi, Pakistan
[2] Department of Endodontics, Baqai Dental College, Karachi, Pakistan
[3] Section of Dentistry, Department of Surgery, Aga Khan University Hospital, Karachi, Pakistan

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):327–334

Address for correspondence
Rashna Hoshang Sukhia
E-mail: rashna_aga@yahoo.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on September 28, 2021
Reviewed on January 10, 2022
Accepted on January 27, 2022

Published online on June 30, 2023

Abstract

This systematic review and meta-analysis aimed to summarize the effectiveness of bonded and removable retainers (the Hawley and Essix retainers) in terms of improving occlusal settling (occlusal contact points/areas) after orthodontic treatment. We searched the Cochrane Library, PubMed, CINAHL Plus, and Dental & Oral Sciences Source (DOSS), as well as SIGLE, Google Scholar and ClinicalTrials.gov for eligible studies. We included randomized and non-randomized controlled trials along with cohort studies. Studies that reported occlusal contacts/areas during retention with fixed bonded and removable retainers were included. To assess the quality of the randomized controlled trials (RCTs), the Cochrane Collaboration risk-of-bias (RoB) tool was utilized, whereas the Newcastle–Ottawa Scale (NOS) was used to assess the quality of cohort studies.

We included 6 articles in our systematic review after scrutinizing 219 articles and eliminating the illegible ones based on duplication, titles, abstracts, and objectives. Bonded retainers (BRs) allowed faster and better posterior occlusal settling as compared to the Hawley retainer (HR). However, HR showed good occlusal settling in the anterior dental arch. The Essix retainer (ER) showed a decrease in occlusal contact during the retention phase. Meta-analysis showed no statistically significant difference between BRs and removable retainers. In conclusion, HR allowed better overall occlusal settling as compared to other retainers. However, BRs allowed faster settling in the posterior tooth region. The Essix retainer showed poor settling of occlusion. Overall, there is an insufficient number of high-quality RCTs to provide additional evidence, and further high-quality RCTs are needed.

Keywords: orthodontic retainers, occlusal contact, vacuum-formed

Cite as
Shoukat Ali U, Zafar K, Hoshang Sukhia R, Fida M, Ahmed A. Effect of bonded and removable retainers on occlusal settling after orthodontic treatment: A systematic review and meta-analysis. *Dent Med Probl.* 2023;60(2):327–334. doi:10.17219/dmp/146194

DOI
10.17219/dmp/146194

Introduction

Retention is considered to be part of orthodontic treatment and plays an important role in post-orthodontic clinical success.[1] Immediate relapse after orthodontic treatment is caused by multiple factors, including the elastic recoil of gingival fibers, malocclusion present before the treatment, the alveolar bone and root condition, differential jaw growth, and unequal pressure from soft tissues.[2] It is crucial to note that it takes nearly 3–4 months for the periodontal ligament (PDL) and 6–12 months for gingival fibers to reorganize and heal. Thenceforth, during this unstable period, occlusal settling via the solid cusp and fossa relationship plays an important role in preventing relapse.[3,4] Among the different factors that need to be controlled after orthodontic treatment, occlusion settling requires no active control and is considered to be a beneficial form of relapse.[5]

Generally, there are two types of retention protocols available, i.e., fixed and removable, which are favored based on the clinician's and patient's choice. The most routinely utilized removable retainers are the Hawley plate and the Essix splint.[6] The Hawley retainer (HR), despite being unesthetic, can be utilized in various clinical situations due to the ease of fabrication and modification. On the other hand, the Essix retainer (ER), which is a vacuum-formed retainer (VFR), may not be very versatile in terms of usage, but is very esthetic and popular among orthodontic patients.[7] Removable retainers also have an inherent disadvantage of dependency on patient compliance, which makes bonded retainers (BRs) an attractive option. Bonded retainers allow the permanent retention of the teeth with minimum patient compliance, provide good esthetics and do not encroach on the occlusal surfaces.[8,9] Considering occlusal settling, conventional HRs and BRs have an advantage over ERs, as they do not cover the occlusal surfaces and allow the passive eruption of the teeth.

Recently, a systematic review compared different aspects of VFR and HR after orthodontic treatment, and found better incisor stability and patient satisfaction with VFR.[10] Similarly, in another systematic review, the authors found BRs to result in good periodontal health and lower incisor stability after orthodontic treatment.[11] However, to the best of our knowledge, no systematic review has compared occlusal settling after debonding between bonded and removable (HR and ER) retainers. The purpose of this systematic review and meta-analysis was to compare BRs with removable retainers (HRs/ERs) in terms of occlusal settling during the retention period.

Material and methods

Protocol and registration

The current systematic review was conducted based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The review protocol was registered at PROSPERO (an international database at the University of York, UK) (CRD42020179410).

Eligibility criteria

Based on our inclusion and exclusion criteria, we included randomized controlled trials (RCTs) and prospective/retrospective cohort studies that assessed the primary outcome.

We followed the PICOS model:

– **P**opulation – patients in the retention phase after orthodontic treatment;
– **I**ntervention – removable retainers (Essix or all Hawley retainer possibilities);
– **C**omparison – bonded retainers (all possibilities of fixed retainers);
– **O**utcome – occlusal contact points/areas (occlusal settling);
– **S**tudy – randomized and non-randomized clinical trials, and cohort studies.

The secondary outcome to be assessed was the improvement in biting force with different retention protocols.

We excluded studies in which participants had any surgical correction involving orthodontic treatment, craniofacial syndrome, failure with compliance, or a retention period of fewer than 2 months.

Information sources and search strategy

A comprehensive literature search was carried out from January 2007 to May 2021 in 4 major health databases (Cochrane Library, PubMed (NLM), CINAHL Plus (EBSCO), and Dental & Oral Sciences Source (DOSS), including hand searches). To identify any grey literature and unpublished data, SIGLE was explored, and a manual search of Google Scholar and the database of www.clinicaltrials.gov was also performed using the following MeSH terms: ("Orthodont*" OR "Dental" OR "Dentist*") AND ("Dental occlusion" OR "Occlusion") AND ("Essix" OR "Hawley" OR "Hawley retainer*" OR "Fixed bonded lingual retainer*" OR "Fixed orthodontic appliance*" OR "Bonded retainer*").

Study selection and data extraction

We included RCTs and prospective/retrospective cohort studies that evaluated the primary outcome. Two authors (USA and AA) independently scrutinized the articles based on their titles and abstracts, and assessed the

eligibility of the studies to be included. Any disagreement between the authors was sorted by consulting a third author (KZ).

Risk of bias in individual studies

For assessing the risk of bias in the RCTs individually, the Cochrane Collaboration tool was used. Terms such as a high risk of bias, an unclear risk of bias and a low risk of bias were assigned to individual RCT studies. The prospective/retrospective cohort studies were assessed for the risk of bias by using the Newcastle–Ottawa Scale (NOS).

Risk of bias across studies

The overall risk of bias for the RCTs was assessed with the Cochrane Collaboration tool. It consists of 7 domains determining the grade of bias risk as a low, unclear or high. The prospective/retrospective cohort studies were assessed for the risk of bias with NOS.

Certainty assessment

To assess the certainty of evidence for occlusal settling (outcome), the Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) approach was applied.

Data analysis

Data analysis was limited to reporting the mean values of occlusal contact points/areas with different retention methods. A meta-analysis was performed for studies with quantitative data, using the RevMan software, v. 5.3 (Cochrane). For the computation of the summary effects, a random effect model was utilized due to high heterogeneity. The I^2 statistics was utilized to assess the heterogeneity among the selected studies.

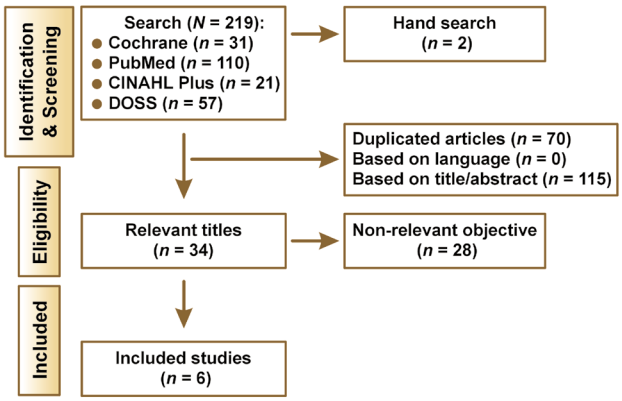

Fig. 1. Study selection PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram

DOSS – Dental & Oral Sciences Source.

Results

Study selection and characteristics

In the initial search of the literature, we found 219 studies across the major databases. After the removal of duplicates, we had 149 articles. Upon scrutinizing the articles based on title and abstract, non-relevant articles were excluded from this systematic review. A total of 6 articles were included – 2 RCTs,[12,13] and 2 prospective[14,15] and 2 retrospective[16,17] cohort studies. The details of the process of study selection are given in the PRISMA flow diagram along with the reasons for the exclusion of a particular study (Fig. 1). The characteristics of the included studies along with their method of outcome assessment are summarized in Tables 1 and 2.

Assessment of the risk of bias within the studies

Randomized controlled trials

We utilized the Cochrane Collaboration risk of bias (RoB) tool to determine the overall and individual risk

Table 1. Studies included in the systematic review

No.	Study	Year	Journal	Sample size	Participants' age [years]	Design
1	Alkan and Kaya[12]	2020	Journal of Oral Rehabilitation	60	N/A	RCT
2	Varga et al[13]	2017	American Journal of Orthodontics and Dentofacial Orthopedics	176	16 (15–18)	RCT
3	Hoybjerg et al.[14]	2013	American Journal of Orthodontics and Dentofacial Orthopedics	90	15.2 (11.1–34.8)	prospective cohort
4	Kara and Yilmaz[15]	2020	American Journal of Orthodontics and Dentofacial Orthopedics	90	18.2 ±7.3	prospective cohort
5	Sari et al.[16]	2009	Angle Orthodontics	70	16.3 ±3.0	retrospective cohort
6	Başçiftçi et al.[17]	2007	American Journal of Orthodontics and Dentofacial Orthopedics	60	HR group: 15.3 ±2.2 FR group: 16.1 ±3.4	retrospective cohort

Data presented as mean ± standard deviation (*M* ±*SD*) or as mean (interquartile range) (*M* (*IQR*)). HR – Hawley retainer; FR – fixed retainer; RCT – randomized clinical trial; N/A – data not available.

Table 2. Characteristics of the included studies

Study	Intervention	Measuring technique	Outcome	Follow-up
Alkan and Kaya[12]	HR/BR/VFR	T-Scan III	OSA and occlusal force distribution	6-month
Varga et al[13]	U/L – ER, U/L –wrap-around retainer and U – ER/L – BR	occlusal force-meter; occlusal contacts were determined using plastic foil	MVBF and NOC	10-week
Hoybjerg et al.[14]	U/L – HR, U – HR/L – BR and U – ER/L –BR	ABO discrepancy index and CRE	alignment/rotation, marginal ridges, buccolingual inclination, overjet, occlusal contacts, occlusal relationship, interproximal contacts, and root angulation	1-year
Kara and Yilmaz[15]	ER group, HR group and BR group	3Shape Ortho Analyzer software	occlusal contact areas and CRE score changes	1-year
Sari et al.[16]	HR and BR	silicon based inter-occlusal registration	occlusal contact points	1-year
Başçiftçi et al.[17]	modified wrap-around HR and maxillary Jensen plate with mandibular FR	silicone-based impression bites	occlusal contact points	1-year

BR – bonded retainer; VFR – vacuum-formed retainer; U – upper arch; L – lower arch; ER – Essix retainer; ABO – American Board of Orthodontics; CRE – cast–radiograph evaluation; OSA – occlusal surface area; MVBF – maximum voluntary bite force; NOC – number of occlusal contacts.

of bias present within the studies. In both studies,[12,13] the blinding of the outcome assessment was not reported; therefore, it was considered a high risk of bias. Similarly, the blinding of participants was not possible due to the nature of the intervention, making it a high risk of bias. The overall risk of bias across the studies was high for the blinding of participants and the outcome assessment. The quality of evidence is summarized in Fig. 2.

Prospective/retrospective cohort studies

The prospective/retrospective cohort studies were assessed for the risk of bias with NOS. All the included studies had a good quality of evidence. The quality of evidence is summarized in Table 3.

Table 3. Assessment of the risk of bias of the included cohort studies, using the Newcastle–Ottawa Scale (NOS)

Study	Selection (4)	Comparability (2)	Outcome (3)	Quality assessment
Hoybjerg et al.[14]	***	*	**	good
Kara and Yilmaz[15]	***	*	**	good
Sari et al.[16]	***	*	**	good
Başçiftçi et al.[17]	***	*	**	good

Good quality: 3 or 4 stars in the selection domain AND 1 or 2 stars in the comparability domain AND 2 or 3 stars in the outcome domain.
Fair quality: 2 stars in the selection domain AND 1 or 2 stars in the comparability domain AND 2 or 3 stars in the outcome domain.
Poor quality: 0 or 1 stars in the selection domain OR 0 stars in the comparability domain OR 0 or 1 stars in the outcome domain.

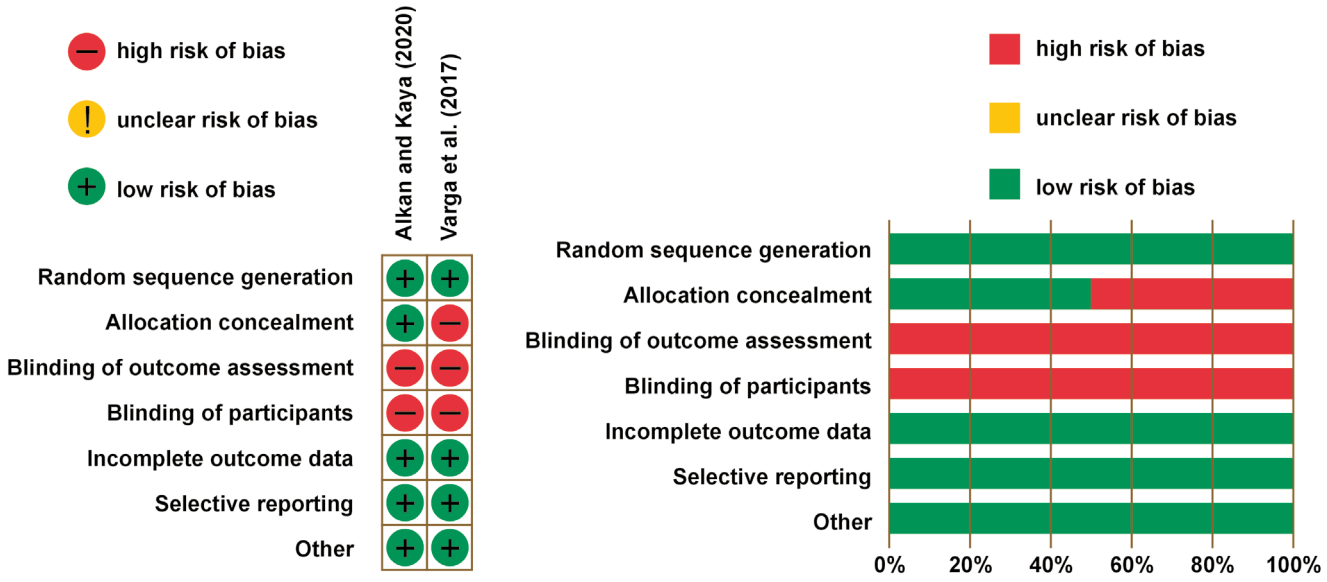

Fig. 2. Assessment of the individual and overall risk of bias of the included randomized controlled trials (RCTs), using the Cochrane Collaboration risk of bias (RoB) tool

Assessment of the certainty level

The certainty of evidence was assessed via the GRADE approach, which demonstrated a low and very low quality of evidence (Table 4).

Results of individual studies

Alkan and Kaya[12] divided the arch into 4 quadrants and reported an increase in the occlusal surface area (OSA) in the left and right posterior dental arches for the HR, BR and VFR groups through a follow-up period of 6 months. However, only HR brought statistically significant improvement in the anterior OSA ($p = 0.008$). Considering the occlusal force distribution, a statistically significant increase was observed in the anterior dental arches for the HR group ($p = 0.026$), whereas in the BR group, better posterior dental occlusal force distribution was reported ($p = 0.029$).

Varga et al.[13] reported an increase in the maximum voluntary bite force (MVBF) and the number of contacts (NOC) in all retainer groups, but the least improvement was observed in subjects with 2 ERs. The type of appliance, age and gender were reported to have a statistically significant impact on NOC ($p = 0.05$).

Hoybjerg et al.[14] reported that the combination of upper HR/lower BR exhibited the best occlusal settling, whereas the upper ER/lower BR combination showed the least amount of settling; however, these differences were statistically non-significant ($p = 0.819$). Similarly, they reported no statistically significant differences between extraction and non-extraction treatment modalities.

Kara and Yilmaz[15] reported a significant increase in occlusal contact areas for all teeth except for incisors in the HR and BR groups ($p < 0.001$). The BR group demonstrated the highest amount of occlusal settling, whereas the ER exhibited poor occlusal settling for the posterior teeth.

Sari et al.[16] reported an increase in the occlusal contact points from 12.45 to 16.40 in the HR group ($p = 0.05$). Similarly, in the BR group, the combined occlusal contacts of all teeth increased by an average of 13.72 ($p < 0.001$). Both groups showed improvement in the posterior occlusal contacts; however, BR showed the greatest improvement.

Başçiftçi et al.[17] reported improvement in the vertical movement of the posterior teeth in both the modified upper/lower HR and upper Jensen/lower BR groups. The BR group showed an increased number of tooth contacts in the posterior segments ($p < 0.001$) as compared to the modified HR group at the end of the 1-year retention period ($p = 0.05$).

Synthesis of the results

We conducted a meta-analysis between BRs and HRs to compare occlusal contacts after debonding. We included 3 comparative studies[12,15,16] in the meta-analysis, which yielded statistically non-significant results. Similarly, in the comparison between BRs and ERs, we included two studies[12,15] and found no statistically significant difference between the 2 groups in terms of occlusal settling. The summary of the effect is depicted in Fig. 3 and 4, showing statistically non-significant results.

Discussion

The current systematic review was conducted to assess the improvement of occlusal contact points/areas in different retention protocols. Recently, a systematic review was also conducted on the effect of removable retainers on tooth stability.[10] However, there is a lack of published systematic reviews investigating the impact of BRs, HRs and ERs on occlusal settling.

Table 4. Summary of findings (Grading of Recommendations, Assessment, Development, and Evaluations (GRADE) approach): Occlusal contact points/areas after orthodontic treatment utilizing different retention protocols (outcome)

Outcome		Starting level of evidence	Risk of bias	Inconsistency	Indirectness	Impreciseness	Reporting bias	Effect	Overall quality of evidence
Occlusal contact points/areas	2 RCTs	high	serious[1]	very serious[2]	no	no	no	all studies reported the worst occlusal settling with ER	very low
	4 cohort studies	low	no	very serious[2]	no	no	no	BR and HR allowed better occlusal settling	low
Improvement in biting force	2 RCTs	high	serious[1]	very serious[2]	no	no	no	BR and HR allowed better biting force	very low

GRADE Working Group grades of evidence*: high quality – further research is very unlikely to change our confidence in the estimate of effect; moderate quality – further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; low quality – further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; very low quality – any estimate of effect is very uncertain.

[1] both studies had a high risk of bias for the blinding of participants and the outcome assessment, similarly, 1 study did not report any concealment method; [2] a high level of heterogeneity is present between the comparison group and the method of assessment.

* Schünemann H, Brożek J, Guyatt G, Oxman A, eds. *GRADE Handbook*. Handbook for grading the quality of evidence and the strength of recommendations using the GRADE approach. GRADE Working Group; 2013.

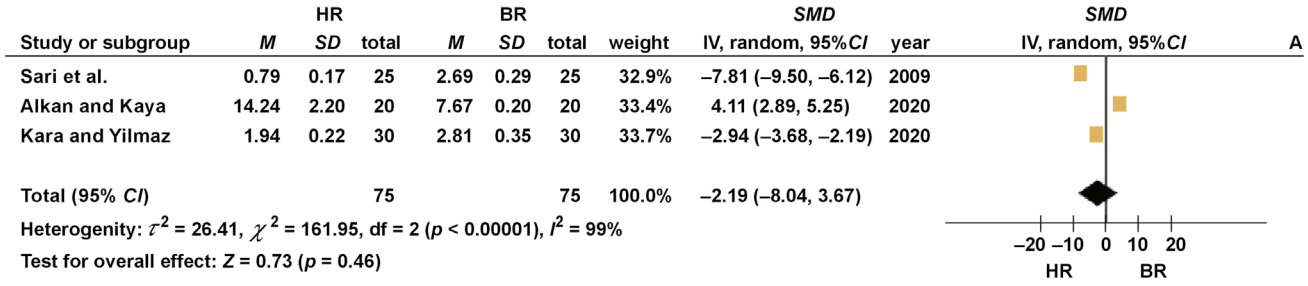

Fig. 3. Forest plot depicting the mean difference (*MD*) between Hawley retainers (HRs) and bonded retainers (BRs)
SMD – standardized mean difference; *CI* – confidence interval; df – degrees of freedom.

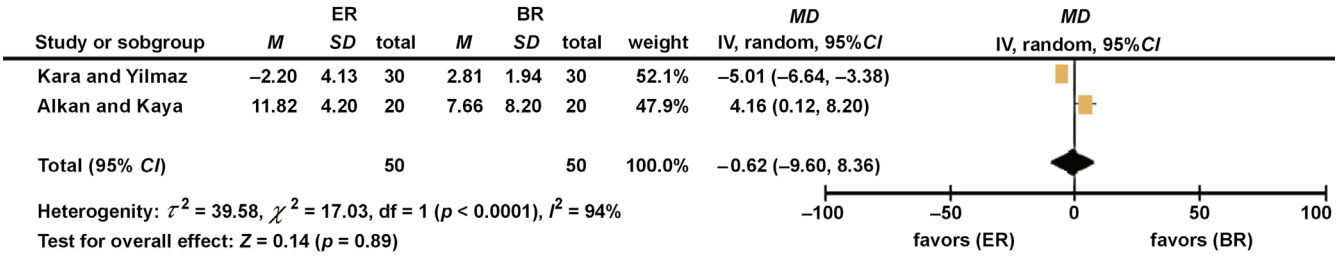

Fig. 4. Forest plot depicting the mean difference (*MD*) between Essix retainers (ERs) and bonded retainers (BRs)

Bonded retainer vs. Hawley retainer

Three studies[12,15,16] reported the improvement of occlusal contacts in the posterior region in both the BR and HR groups. However, BRs performed better in terms of efficient occlusal settling of the posterior teeth. Considering the anterior occlusal contacts, only one study[12] reported a statistically significant increase in the HR group as compared to the BR group. Other studies[15,16] failed to find any difference between the groups with regard to improvement in the anterior occlusal contacts. These results are in accordance with other studies, which found no significant improvement in the anterior occlusal contacts when using HRs.[18,19] Considering the secondary outcome, better occlusal force distribution was observed in the anterior region for the HR group as compared to the BR group. Contrary to this, the BR group showed a considerable decline in anterior occlusal forces, whereas posterior occlusal forces were higher as compared to the HR group.[12] Similarly, 2 studies[14,15] evaluated the retainer protocols based on the cast–radiograph evaluation (CRE), which was established by the American Board of Orthodontics (ABO) to assess the excellence of occlusion for board certification. In terms of CRE scores, the HR group performed particularly well in contrast to the BR group. The HR group exhibited the best post-treatment results with improvement in 5 criteria and demonstrated a statistically significant decrease in the total CRE scores.[15] This is in agreement with the study conducted by Aslan et al., who reported a reduction in the CRE scores with HR.[20]

Bonded retainer vs. Essix retainer

Conflicting results were found when comparing BR with ER. Varga et al.[13] found correlations between a reduced NOC and female gender and the ER protocol. On the contrary, Kara and Yilmaz[15] reported statistically significant improvement in occlusal contact, the marginal ridge and the overjet in the ER group, as determined by the CRE scores, but found a reduced OSA based on the OrthoAnalyzer software analysis. The authors found a reduced OSA in the ER group except for incisors as compared to the BR group, in which anterior occlusal contacts were found to be less.[15] When assessing VFRs, Sauget et al. reported no significant increases in the anterior and posterior occlusal contacts,[19] whereas Aslan et al. reported improved anterior occlusal contacts only.[20] However, when comparing the CRE scores of the BR and ER groups, one study[15] found BRs to be superior, although it was statistically non-significant. On the other hand, Hoybjerg et al.[14] reported a decrease in the total CRE scores with the Essix appliance. Alkan and Kaya[12] reported a statistically significant increase in OSA in the ER and BR groups. However, the ER group outperformed the BR group when anterior occlusal contacts were evaluated after 6 months of full-time wear. Interestingly, the wear time had no influence on anterior occlusal contacts, as one author[15] divided the wear time of ER equally into 6 months of full-time followed by nighttime wear only and found the same results at the end of a 1-year evaluation.

Combination of different retainers

Hoybjerg et al.[14] evaluated occlusal contacts in different retainer patterns, i.e., upper/lower HR, upper HR/lower BR and upper ER/lower BR. The authors reported that the best combination of retainers for reducing the CRE scores were upper HR/lower BR followed by upper/lower HR and upper ER/lower BR. In terms of occlusal contacts, each retainer combination showed a statistically significant reduction in the CRE scores (improvement in occlusal contacts) at 1-year follow-up.[14] This is in line with a study that reported a decrease in the CRE scores during retention and improvement in certain aspects of occlusion, i.e., the overjet, occlusal contacts and the marginal ridge.[21] Greco et al. reported increased CRE scores after the retention period with fixed and removable retainers.[22] However, in terms of occlusal settling, the upper HR/lower BR group showed the highest amount of occlusal settling.[14] The same authors evaluated the impact of extraction and non-extraction treatment on occlusal contacts, and reported no statistical difference between them. Yet, based on the initial severity of discrepancy, they reported a statistically significant difference between low and high discrepancy groups.[14] Başçiftçi et al.[17] compared upper Jensen plate/lower fixed bonded retainer to upper/lower modified HRs. They showed the same trend of faster and improved posterior occlusal contacts in the BR group as compared to the HR group.[17]

Outcome evaluation methods across different studies

One of the important considerations are the outcome evaluation methods used in the included studies for assessing the occlusal contact points/areas. More traditional methods of inter-occlusal registration via silicon-based impression material were utilized by 2 included studies.[16,17] These inter-occlusal registrations are evaluated under light to determine NOC. In the preceding literature, this is the most common and practical method to assess occlusal contacts.[23,24] This method might not be ideal, since light transmittance angles can influence the outcome. On the other hand, Varga et al.[13] assessed occlusal contacts by using plastic foil, which was 6 mm in width and 0.05 mm in thickness. The authors took into account contact points in areas where the foil could not be pulled out by heavy pulling during habitual occlusion.[13] The previously recorded error of this method is 10% of the mean value.[25] However, assessing occlusal contacts by utilizing silicone-based impression and plastic foil materials may provide inadequate information about posterior occlusal contacts. One study[14] utilized the ABO grading system, and assessed pre- and post-treatment occlusal contacts on orthodontic cast models, as recommended by Casko et al.[26] Two of the included studies[12,15] used modern digital technology to assess occlusal contact areas in their sample subjects. T-scan III was utilized by Alkan and Kaya,[12] who reported difficulty in the assessment of occlusal contacts, so they preferred recording OSA. This method has a low degree of validity for quantifying absolute force, but demonstrates a high degree of reliability in consecutive measurements.[27] These contemporary systems have been developed to accurately and precisely evaluate occlusal forces and contacts; however, they have some disadvantages as well. The pressure-sensitive plates used in dental prescale systems are 0.004-inch-thick, and due to the impenetrable nature of the plate, the overdetection of OSA is reported near the hinge axis.[28] Similarly, occlusal three-dimensional (3D) anatomy might not be fully captured, as in silicon or wax bite materials. To overcome these shortcomings, Kara and Yilmaz[15] scanned the pre- and post-treatment models with a 3D laser scanner (3Shape) separately in an occluded manner. To measure the occlusal region, the screenshots of each tooth were transferred to the OrthoAnalyzer software and two-dimensional (2D) area measurements were taken. This method has been found to be both reliable and repeatable.[29]

The risk of bias across RCTs was assessed with the help of the Cochrane Collaboration RoB tool. Both RCTs[12,13] performed randomizations, but provided no information on the method utilized. Similarly, Varga et al.[13] did not provide any information on allocation concealment. The risk of bias for the cohort studies was assessed with NOS, and each included study[14–17] demonstrated a good quality of evidence.

Limitations

The limitations of the present systematic review involve heterogeneity in the assessment of occlusal contact points/areas, a limited number of RCTs, different combinations of retainers, and different evaluation intervals for the assessment of occlusal contacts. Few of the studies[12,15] assessed OSA, which represents the functional state of the occlusal table, whereas other studies[13,14,16,17] reported occlusal contact points, which represent the static nature of occlusion. Similarly, retention follow-ups were a maximum of 1 year, which is not sufficient to conclude any strong recommendation regarding the retention protocols in review.

Conclusions

In conclusion, HRs performed well, particularly in the anterior segment of the arch, as compared to BRs, whereas BRs allowed better and faster occlusal settling in the posterior arch. The Essix retainer showed poor occlusal settling in the posterior region. As for the maximum biting force, HRs performed better in the anterior region and BRs in the posterior region.

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

Umair Shoukat Ali https://orcid.org/0000-0001-9252-8934
Kamil Zafar https://orcid.org/0000-0003-0060-8115
Rashna Hoshang Sukhia https://orcid.org/0000-0001-9210-6432
Mubassar Fida https://orcid.org/0000-0003-4842-9896
Aqeel Ahmed https://orcid.org/0000-0003-0316-7546

References

1. Little RM, Riedel RA, Stein A. Mandibular arch length increase during the mixed dentition: Postretention evaluation of stability and relapse. *Am J Orthod Dentofacial Orthop*. 1990;97(5):393–404. doi:10.1016/S0889-5406(08)70111-O
2. Littlewood SJ, Kandasamy S, Huang G. Retention and relapse in clinical practice. *Aust Dent J*. 2017;62(Suppl 1):51–57. doi:10.1111/adj.12475
3. Johnston CD, Littlewood SJ. Retention in orthodontics. *Br Dent J*. 2015;218(3):119–122. doi:10.1038/sj.bdj.2015.47
4. Martin M. Preconditions for long-term stability of treatment. *J Dentofacial Anom Orthod*. 2016;19(1):103. doi:10.1051/odfen/2015029
5. Sullivan B, Freer TJ, Vautin D, Basford KE. Occlusal contacts: Comparison of orthodontic patients, posttreatment patients, and untreated controls. *J Prosthet Dent*. 1991;65(2):232–237. doi:10.1016/0022-3913(91)90167-u
6. Demir A, Babacan H, Nalcacı R, Topcuoglu T. Comparison of retention characteristics of Essix and Hawley retainers. Korean J *Orthod*. 2012;42(5):255–262. doi:10.4041/kjod.2012.42.5.255
7. Valiathan M, Hughes E. Results of a survey-based study to identify common retention practices in the United States. *Am J Orthod Dentofacial Orthop*. 2010;137(2):170–177. doi:10.1016/j.ajodo.2008.03.023
8. Al-Moghrabi D, Pandis N, Fleming PS. The effects of fixed and removable orthodontic retainers: A systematic review. *Prog Orthod*. 2016;17(1):24. doi:10.1186/s40510-016-0137-x
9. Littlewood SJ, Russell JS, Spencer RJ. Why do orthodontic cases relapse? *Orthod Update*. 2009;2(2):38–44. doi:10.12968/ortu.2009.2.2.38
10. Outhaisavanh S, Liu Y, Song J. The origin and evolution of the Hawley retainer for the effectiveness to maintain tooth position after fixed orthodontic treatment compare to vacuum-formed retainer: A systematic review of RCTs. *Int Orthod*. 2020;18(2):225–236. doi:10.1016/j.ortho.2020.02.008
11. Arn ML, Dritsas K, Pandis N, Kloukos D. The effects of fixed orthodontic retainers on periodontal health: A systematic review. *Am J Orthod Dentofacial Orthop*. 2020;157(2):156–164. e17. doi:10.1016/j.ajodo.2019.10.010
12. Alkan Ö, Kaya Y. Changes in occlusal surface area and occlusal force distribution following the wear of vacuum-formed, Hawley and bonded retainers: A controlled clinical trial. J *Oral Rehabil*. 2020;47(6):766–774. doi:10.1111/joor.12970
13. Varga S, Spalj S, Milosevic SA, et al. Changes of bite force and occlusal contacts in the retention phase of orthodontic treatment: A controlled clinical trial. *Am J Orthod Dentofacial Orthop*. 2017;152(6):767–777. doi:10.1016/j.ajodo.2017.03.028
14. Hoybjerg AJ, Currier GF, Kadioglu O. Evaluation of 3 retention protocols using the American Board of Orthodontics cast and radiograph evaluation. *Am J Orthod Dentofacial Orthop*. 2013;144(1):16–22. doi:10.1016/j.ajodo.2013.02.022
15. Kara B, Yilmaz B. Occlusal contact area changes with different retention protocols: 1-year follow-up. *Am J Orthod Dentofacial Orthop*. 2020;157(4):533–541. doi:10.1016/j.ajodo.2019.05.020
16. Sari Z, Uysal T, Başçiftçi FA, Inan O. Occlusal contact changes with removable and bonded retainers in a 1-year retention period. *Angle Orthod*. 2009;79(5):867–872. doi:10.2319/101608-536.1
17. Başçiftçi FA, Uysal T, Sari Z, Inan O. Occlusal contacts with different retention procedures in 1-year follow-up period. *Am J Orthod Dentofacial Orthop*. 2007;131(3):357–362. doi:10.1016/j.ajodo.2005.05.052
18. Durbin DS, Sadowsky C. Changes in tooth contacts following orthodontic treatment. *Am J Orthod Dentofacial Orthop*. 1986;90(5):375–382. doi:10.1016/0889-5406(86)90003-x
19. Sauget E, Covell DA Jr., Boero RP, Lieber WS. Comparison of occlusal contacts with use of Hawley and clear overlay retainers. *Angle Orthod*. 1997;67(3):223–230. doi:10.1043/0003-3219(1997)067<0223:COOCWU>2.3.CO;2
20. Aslan BI, Dinçer M, Salmanli O, Qasem MA. Comparison of the effects of modified and full-coverage thermoplastic retainers on occlusal contacts. *Orthodontics (Chic.)*. 2013;14(1):e198–e208. doi:10.11607/ortho.990
21. Nett BC, Huang GJ. Long-term posttreatment changes measured by the American Board of Orthodontics objective grading system. *Am J Orthod Dentofacial Orthop*. 2005;127(4):444–450. doi:10.1016/j.ajodo.2004.03.029
22. Greco PM, English JD, Briss BS, et al. Posttreatment tooth movement: For better or for worse. *Am J Orthod Dentofacial Orthop*. 2010;138(5):552–558. doi:10.1016/j.ajodo.2010.06.002
23. Dinçer M, Aslan BI. Effects of thermoplastic retainers on occlusal contacts. *Eur J Orthod*. 2010;32(1):6–10. doi:10.1093/ejo/cjp062
24. Dinçer M, Meral O, Tümer N. The investigation of occlusal contacts during the retention period. *Angle Orthod*. 2003;73(6):640–646. doi:10.1043/0003-3219(2003)073<0640:TIOOCD>2.0.CO;2
25. Bakke M, Michler L. Temporalis and masseter muscle activity in patients with anterior open bite and craniomandibular disorders. *Scand J Dent Res*. 1991;99(3):219–228. doi:10.1111/j.1600-0722.1991.tb01888.x
26. Casko JS, Vaden JL, Kokich VG, et al. Objective grading system for dental casts and panoramic radiographs. American Board of Orthodontics. *Am J Orthod Dentofacial Orthop*. 1998;114(5):589–599. doi:10.1016/s0889-5406(98)70179-9
27. Cerna M, Ferreira R, Zaror C, Navarro P, Sandoval P. Validity and reliability of T-Scan(®) III for measuring force under laboratory conditions. *J Oral Rehabil*. 2015;42(7):544–551. doi:10.1111/joor.12284
28. Ando K, Fuwa Y, Kurosawa M, Kondo T, Goto S. Bite force measurement system using pressure-sensitive sheets and silicon impression materials. *Dent Mater J*. 2009;28(2):212–218. doi:10.4012/dmj.28.212
29. Almasoud N, Bearn D. Little's irregularity index: Photographic assessment vs study model assessment. *Am J Orthod Dentofacial Orthop*. 2010;138(6):787–794. doi:10.1016/j.ajodo.2009.01.031

Review

Therapies for sleep bruxism in dentistry: A critical evaluation of systematic reviews

Lissette Cerón[1,A–D], Mishelle Pacheco[1,A–D], Andrés Delgado Gaete[2,A,C], Wilson Bravo Torres[1,E,F], Daniela Astudillo Rubio[1,E]

[1] Graduate Program in Oral Rehabilitation and Implant-Assisted Prosthetics, Faculty of Dentistry, University of Cuenca (Universidad de Cuenca), Ecuador
[2] Department of Prostodontics, School of Dentistry, Catholic University of Cuenca (Universidad Católica de 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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):335–344

Address for correspondence
Lissette Cerón
E-mail: lizycm993@hotmail.com

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on October 27, 2022
Reviewed on November 7, 2022
Accepted on November 9, 2022

Published online on November 28, 2022

Abstract

The aim of the study was to evaluate the methodological quality and the risk of bias of systematic reviews with regard to the literature on therapies for sleep bruxism (SB) in dentistry, applying the AMSTAR 2 (A MeaSurement Tool to Assess systematic Reviews) qualitative guide, as well as the effectiveness of various kinds of treatment of SB. Initially, a total of 1,499 articles were obtained from 4 databases and 2 websites. Relevant articles were obtained from the PubMed, Scopus, Cochrane, and Embase databases as well as from Google Scholar and OpenGrey. Six systematic reviews that met the eligibility criteria were included. The methodological quality of all systematic reviews, assessed with the AMSTAR 2 tool, was critically low. Regarding treatment effectiveness, 5 systematic reviews reported on pharmacological management (botulinum toxin type A (BTX-A), clonazepam and clonidine), 2 reported on oral appliances (OAs) (stabilizing splints and mandibular advancement devices (MADs)) and 1 study addressed the effects of biofeedback (BF). The results of the therapies were diverse and confusing. The available research is not conclusive, and does not show clear evidence or a consensus on the part of researchers on the most effective treatment for the management of SB. More research of better methodological quality is needed in this area.

Keywords: treatment, sleep bruxism, teeth grinding, rhythmic masticatory muscle activity

Cite as
Cerón L, Pacheco M, Delgado Gaete A, Bravo Torres W, Astudillo Rubio D. Therapies for sleep bruxism in dentistry: A critical evaluation of systematic reviews. *Dent Med Probl.* 2023;60(2):335–344. doi:10.17219/dmp/156400

DOI
10.17219/dmp/156400

Introduction

For decades, the term 'bruxism' has generated controversy in the academic and professional environment of dentistry due to the various definitions attributed to it and its alleged association with etiological factors that are currently considered to have no scientific relevance.

Various conceptualizations have been postulated for the definition of bruxism. Lobbezzo et al. in the 2013 international consensus defines sleep bruxism (SB) as "repetitive jaw-muscle activity characterized by clenching or grinding of the teeth and/or bracing or thrusting of the mandible",[1] the International Classification of Sleep Disorders – Third Edition (ICSD-3) classifies it as a sleep-related movement disorder,[2] and Lobbezoo et al. in the 2018 international consensus updated the above definition of bruxism with other findings: (i) suggested separate definitions for SB and awake bruxism, the same being the chewing muscle activity that occurs during sleep (characterized as rhythmic or non-rhythmic) and during wakefulness (characterized by repetitive or sustained tooth contact and/or jaw effort or thrust); (ii) stated that bruxism should not be considered as a disorder, but as a behavior that may be a risk factor (and/or a protective factor) for certain clinical consequences in healthy individuals; and (iii) grouped the techniques for the diagnosis of bruxism into non-instrumental (self-reports) and instrumental (electromyography and polysomnography).[3] Regarding the etiology of bruxism, in the past, it was associated with occlusal discrepancies, but nowadays, it is no longer considered as such,[4] since several studies mention that SB is centrally regulated.[1,5] Despite these changes, the hypothesis of occlusal discrepancies has not been completely abandoned, which has led to confusion among clinicians in terms of making an accurate diagnosis, and therefore applying effective treatment – botulinum toxin type A (BTX-A),[6–8] oral appliances (OAs),[9] biofeedback (BF),[10,11] physical therapy,[12,13] or pharmacotherapy.[14] Still, the efficacy of some of the abovementioned therapies for the management of bruxism has not been scientifically proven. There should be more randomized controlled clinical studies; in some cases, the authors even suggest conducting studies with larger samples and longer treatment periods to obtain results that would be reliable for clinical application. For this reason, as researchers, we feel the need to try to establish which therapies are really valid for the management of SB.

We have not found general studies that would evaluate the methodological quality of systematic reviews on this topic; therefore, this study will surely become a reference for future research. The aim of the present study was to evaluate the methodological quality of the literature and the risk of bias in the systematic reviews addressing therapies for SB by applying the AMSTAR 2 (A MeaSurement Tool to Assess systematic Reviews) qualitative guide[15] as well as to assess the effectiveness of the therapies in terms of their clinical application. The research question was as follows: What is the methodological quality of studies analyzing the treatment of sleep bruxism and what is the effectiveness of various kinds of treatment?

Material and methods

Protocol and registration

This study was carried out in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) 2020 Statement,[16] and a general protocol based on the INPLASY (International Platform of Registered Systematic Review and Meta-analysis Protocols) guide for the registration of systematic review protocols (2021)[17] was also executed. The record is publicly available under number 2021100080 and doi:10.37766/inplay2021.10.0080.

Eligibility criteria

The included studies were systematic reviews, with or without a meta-analysis, that evaluated the different kinds of treatment used in adult patients (aged 18 years or above) diagnosed with bruxism through polysomnography, electromyography and self-reports. No time or language restrictions were applied.

The exclusion criteria embraced literature reviews, intervention studies, observational studies, in vitro laboratory research, randomized controlled clinical studies, abstracts, comments, case reports, protocols, personal opinions, expert opinions, letters, and posters. If a particular article of interest was not available, the author was contacted via e-mail; if after 3 attempts within an interval of 30 days there was no response, the study was excluded. In addition, studies in which methodology was not addressed, or studies using unspecified or non-validated diagnostic methods (self-reports) were also excluded.

Search strategy

An electronic search was conducted in February 2022 in 4 databases (PubMed, Scopus, Cochrane, and Embase). The gray literature was also searched through Google Scholar and OpenGrey. The retrieved articles were exported to a web application (Mendeley) and duplicate articles were eliminated. The search strategy used for each source of information is provided in Table 1. Study selection was carried out in 2 phases: phase 1 consisted of reading the title and the abstract; and phase 2 consisted of reading the full text (Fig. 1).

The aforementioned procedures were performed by 2 reviewers independently, and in case of disagreement, a third reviewer was consulted.

Table 1. Search strategy used for each of the sources of information

Source of information	Search strategy
PubMed	(""bruxism"" [MeSH Terms] OR ""sleep bruxism"" [MeSH Terms]) AND (((""systematic review"" [Publication Type] OR ""systematic review as topic"" [MeSH Terms] OR ""systematic review"" [All Fields]) OR (""review"" [Publication Type] OR ""literature review as topic"" [MeSH Terms] OR ""review"" [All Fields]) AND (""publications"" [MeSH Terms] OR ""literature"" [MeSH Terms])) OR (""literature review as topic"" [MeSH Terms]))
Scopus	(TITLE-ABS-KEY ("bruxism sleep") OR TITLE-ABS-KEY ("bruxism") AND TITLE-ABS-KEY ("systematic review"))
Cochrane	("systematic review" OR "systematic reviews" OR "systematic literature review" OR "systematic literature reviews" OR "meta analysis" OR "meta synthesis" OR "systematic" OR "review" OR "reviews") AND ("bruxism" OR "sleep bruxism" OR "bruxist") AND ("therapy" OR "adult" OR "treatment" OR "effectiveness") in Title Abstract Keyword
Embase	('bruxism'/exp OR 'bruxism' OR 'sleep bruxism'/exp OR 'sleep bruxism' OR 'awake bruxism') AND ('systematic review'/exp OR 'systematic review' OR 'integrative review'/exp OR 'integrative review' OR 'meta-analysis'/exp OR 'meta analysis' OR 'overview' OR 'review'/exp OR 'review' OR 'systematic literature review' OR 'rapid review'/exp OR 'rapid review')
Google Scholar	"treatment bruxism" AND "systematic review"
OpenGrey	"treatment" AND "bruxism"

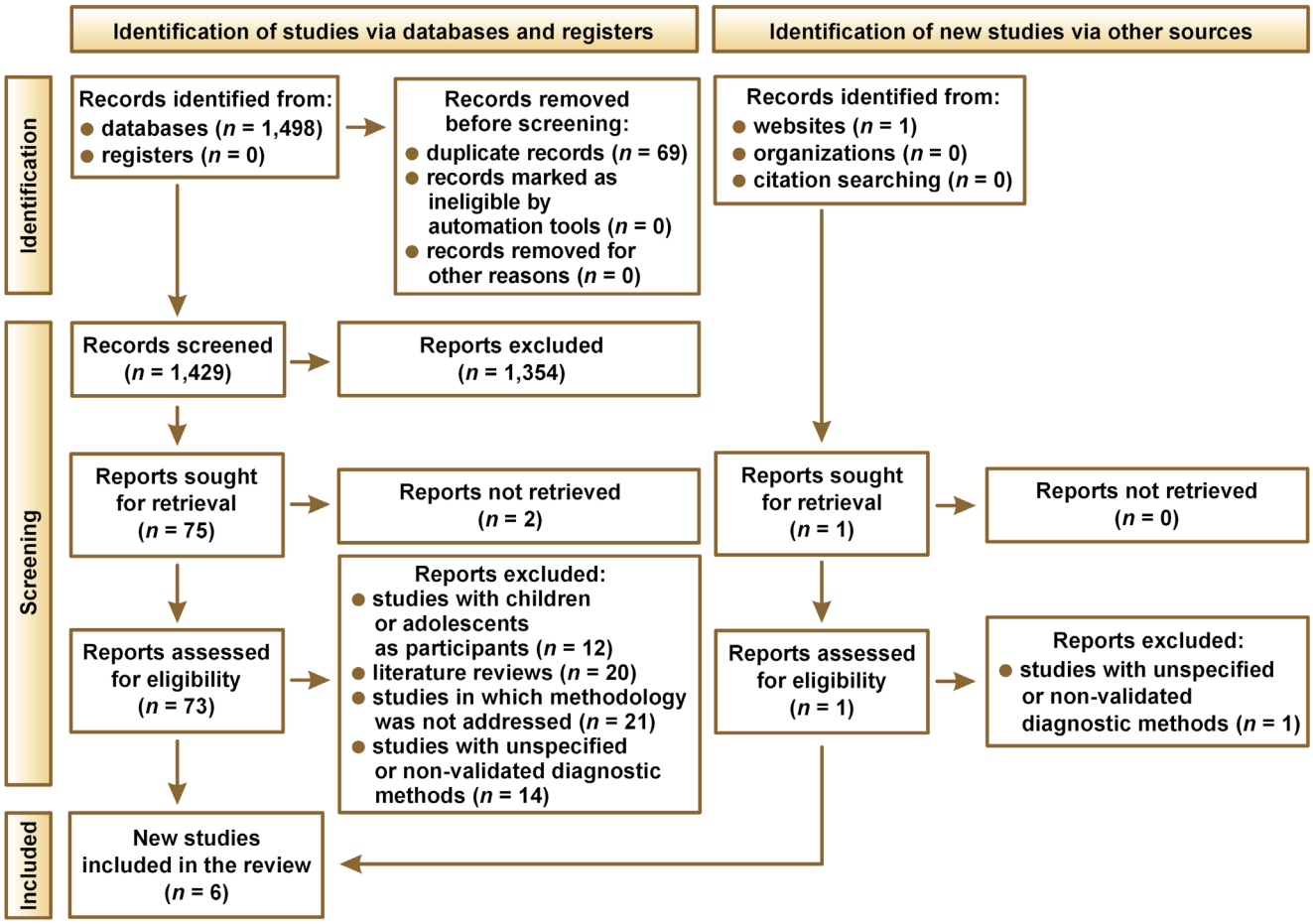

Fig. 1. Study selection flowchart

Data collection process and data elements

Once duplicate studies were eliminated, 2 reviewers independently compiled the data in a table. Any disagreement was resolved by a third reviewer. After the selection of articles, the following information was extracted: author; year of publication; journal and its impact factor; population; interventions and comparators; design of the primary studies; and diagnostic methods for SB (Table 2).

Evaluation of methodological quality, quality of evidence and meta-bias

The evaluation of the methodological quality of the 6 included systematic reviews was performed by 2 reviewers independently by means of the AMSTAR 2 qualitative guide.[15]

The 6 systematic reviews were evaluated according to the PRISMA 2020 Statement,[16] which consists of 27 items.

Table 2. Summary of the overall descriptive characteristics of the included systematic reviews

Author, year, country, journal with its IF	Population	Interventions (I) and comparators (C)	Primary study design	Diagnostic methods for bruxism
Ågren et al.[18] 2020, Sweden *J Oral Rehabil* IF = 3.837	bruxism patients	BTX-A (I) placebo (C)	RCT, prospective or retrospective studies	instrumental approaches
Fernández-Núñez et al.[8] 2019, Spain *Med Oral Patol Oral Cir Bucal* IF = 2.555	bruxism patients	BTX-A (I) placebo, occlusal splints, medications or cognitive-behavioral therapy (C)	RCT	instrumental and non-instrumental approaches
Manfredini et al.[19] 2015, Italy *J Oral Rehabil* IF = 3.837	bruxism patients	SB diagnosis (I) the comparison was based on the description of the condition and features of the passive or active control group	RCT and uncontrolled before–after studies	instrumental approaches
Jokubauskas et al.[20] 2018, Lithuania *J Oral Rehabil* IF = 3.837	bruxism patients	occlusal splints and MADs (I) nociceptive trigeminal inhibitory splint, BF (C)	RCT, before–after crossover	instrumental approaches
Long et al.[23] 2012, China *Int Dent J* IF = 2.512	bruxism patients	BTX-A (I) placebo or other interventional procedures (C)	RCT and non-randomized studies	instrumental and non-instrumental approaches
De la Torre Canales et al.[24] 2017, Brazil *Clin Oral Investig* IF = 3.623	bruxism patients	BTX-A (I) other treatment (C)	RCT, prospective and before–after	instrumental and non-instrumental approaches

IF – impact factor; BTX-A – botulinum toxin type A; SB – sleep bruxism; MADs – mandibular advancement devices; BF – biofeedback; RCT – randomized controlled trial.

Data synthesis

The main results of the included systematic reviews were summarized, and only the primary studies that evaluated a decrease in the electromyographic activity with the use of instrumental tools were analyzed according to each treatment applied, discarding those whose diagnostic method was non-instrumental. A visual indication system (traffic light) was used, where green represented treatment with the best results, red represented treatment with the worst results, and yellow indicated that there were no differences between the compared groups (Table 3).

Results

Review and selection of the primary studies

The search in the electronic databases identified 1,498 studies published between 2012 and 2022; after duplicates were eliminated, 1,431 remained. In addition, 1 article was found in the gray literature. In phase 1, the title and the abstract were reviewed, and 140 articles were selected; in phase 2, the texts were read in full, obtaining 18 articles, of which 6 systematic reviews were included for the qualitative synthesis. A total of 12 primary studies were identified within the systematic reviews. All systematic reviews were rated as critically low according to the AMSTAR 2 tool.[15] More information on the evaluation of methodological quality can be found in Table 4.

Report on main findings

Of the 6 systematic reviews, 5 reported on the pharmacological management of bruxism. Four of them addressed the application of botulinum toxin type A (BTX-A), either into the temporalis or masseter muscle, unilaterally or bilaterally, with doses ranging from 8 IU to 80 IU. The information available on this topic is not conclusive, and although the results of some studies support the effectiveness of BTX-A in reducing the intensity of episodes of bruxism, there is not enough evidence to recommend this drug for the treatment of bruxism. One systematic review compared the effectiveness of clonazepam and clonidine with respect to placebo, showing a reduction in the episodes of bruxism; however, the follow-up period was limited.

Two systematic reviews reported on the effectiveness of OAs; comparison groups treated with stabilization splints and mandibular advancement devices (MADs) were included, and intermittent vs. continuous use and design were analyzed. The results of these investigations suggest that stabilization splints for intermittent use are the most recommended. Regarding MADs, both studies agree that they can significantly reduce bruxism and improve sleep quality, but can also cause muscle pain and temporomandibular disorders (TMDs).

Table 3. Results regarding the efficacy of therapies for sleep bruxism (SB) represented graphically by color, with green and red representing the best and the worst treatment, respectively, and yellow indicating that there were no differences between the compared groups

Systematic review	Primary study	Treatment	Intervention group (IG)	Control group (CG)	Reported results	Diagnostic method
Ågren et al.[18] Fernández-Núñez et al.[8] Manfredini et al.[19] Long et al.[23] De la Torre Canales et al.[24]	Lee SJ, McCall WD Jr., Kim YK, Chung SC, Chung JW. Effect of botulinum toxin injection on nocturnal bruxism: A randomized controlled trial. *Am J Phys Med Rehabil*. 2010;89(1):16–23. doi:10.1097/PHM.0b013e3181bc0c78	BTX-A			$p < 0.001$* for IG as compared to placebo (CG)	EMG
Ågren et al.[18]	Ondo WG, Simmons JH, Shahid MH, Hashem V, Hunter C, Jankovic J. Onabotulinum toxin-A injections for sleep bruxism: A double-blind, placebo-controlled study. *Neurology*. 2018;90(7):e559–e564. doi:10.1212/WNL.0000000000004951				$p = 0.090$ for both IG and placebo (CG)	EMG
Manfredini et al.[19] De la Torre Canales et al.[24]	Shim YJ, Lee MK, Kato T, Park HU, Heo K, Kim ST. Effects of botulinum toxin on jaw motor events during sleep in sleep bruxism patients: A polysomnographic evaluation. *J Clin Sleep Med*. 2014;10(3):291–298. doi:10.5664/jcsm.3532				$p < 0.001$* for both IG and placebo (CG)	EMG
Manfredini et al.[19]	Sato M, Iizuka T, Watanabe A, et al. Electromyogram biofeedback training for daytime clenching and its effect on sleep bruxism. *J Oral Rehabil*. 2015;42(2):83–89. doi:10.1111/joor.12233	BF			$p < 0.050$* for IG as compared to placebo (CG)	EMG
	Valiente López M, van Selms MK, van der Zaag J, Hamburger HL, Lobbezoo F. Do sleep hygiene measures and progressive muscle relaxation influence sleep bruxism? Report of a randomised controlled trial. *J Oral Rehabil*. 2015;42(4):259–265. doi:10.1111/joor.12252	sleep hygiene instructions and Jacobson's relaxation techniques			$p > 0.050$ for both IG and information on the condition of SB (CG)	PSG
	Saletu A, Parapatics S, Anderer P, Matejka M, Saletu B. Controlled clinical, polysomnographic and psychometric studies on differences between sleep bruxers and controls and acute effects of clonazepam as compared with placebo. *Eur Arch Psychiatry Clin Neurosci*. 2010;260(2):163–174. doi:10.1007/s00406-009-0034-0	clonazepam			$p = 0.010$* for IG as compared to placebo (CG)	PSG
	Carra MC, Macaluso GM, Rompré PH, et al. Clonidine has a paradoxical effect on cyclic arousal and sleep bruxism during NREM sleep. *Sleep*. 2010;33(12):1711–1716. doi:10.1093/sleep/33.12.1711	clonidine			$p = 0.020$* for IG as compared to placebo (CG)	PSG
	Madani AS, Abdollahian E, Khiavi HA, et al. The efficacy of gabapentin versus stabilization splint in management of sleep bruxism. *J Prosthodont*. 2013;22(2):126–131. doi:10.1111/j.1532-849X.2012.00914.x	hard stabilization splint			$p < 0.050$* for both IG and gabapentin (CG)	PSG
	Matsumoto H, Tsukiyama Y, Kuwatsuru R, Koyano K. The effect of intermittent use of occlusal splint devices on sleep bruxism: A 4-week observation with a portable electromyographic recording device. *J Oral Rehabil*. 2015;42(4):251–258. doi:10.1111/joor.12251	stabilization splint for continuous use			$p < 0.050$* for intermittent use (CG) as compared to continuous use (IG)	EMG
Jokubauskas et al.[20]	Dalewski B, Chruściel-Nogalska M, Frączak B. Occlusal splint versus modified nociceptive trigeminal inhibition splint in bruxism therapy: A randomized, controlled trial using surface electromyography. *Aust Dent J*. 2015;60(4):445–454. doi:10.1111/adj.12259	mandibular occlusal splints			$p > 0.050$ for both IG and modified nociceptive trigeminal inhibitory splint (CG)	EMG
	Singh PK, Alvi HA, Singh BP, et al. Evaluation of various treatment modalities in sleep bruxism. *J Prosthet Dent*. 2015;114(3):426–431. doi:10.1016/j.prosdent.2015.02.025	reinforced adjustable MADs			$p > 0.050$ for both IG and occlusal splints (CG)	PSG
	Gu WP, Yang J, Zhang FM, Yin XM, Wei XL, Wang C. Efficacy of biofeedback therapy via a mini wireless device on sleep bruxism contrasted with occlusal splint: A pilot study. *J Biomed Res*. 2015;29(2):160–168. doi:10.7555/JBR.28.20130145	maxillary occlusal splint and vibratory feedback			$p = 0.001$* for IG as compared to maxillary occlusal splint without vibration (CG)	PSG

EMG – electromyography; PSG – polysomnography; * statistically significant.

Table 4. AMSTAR 2 (A MeaSurement Tool to Assess systematic Reviews) – critical appraisal tool for systematic reviews that include randomized or non-randomized studies of healthcare interventions, or both

Systematic review	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Overall confidence
Ågren et al.[18] 2020											×	×			×		critically low
Fernández-Núñez et al.[8] 2019											×	×			×		critically low
Manfredini et al.[19] 2015											×	×			×		critically low
Jokubauskas et al.[20] 2018											×	×			×		critically low
Long et al.[23] 2012											×	×			×		critically low
De la Torre Canales et al.[24] 2017											×	×			×		critically low

Answers marked as colors: green – yes; yellow – partial yes; red – no; green-gray – no meta-analysis (×).
Questions:
Q1: Did the research questions and the inclusion criteria for the review comprise the components of PICO?
Q2: Did the report of the review contain an explicit statement that the review methods had been established prior to conducting the review, and did the report justify any significant deviations from the protocol?*
Q3: Did the review authors explain their selection of the study designs for inclusion in the review?
Q4: Did the review authors use a comprehensive literature search strategy?*
Q5: Did the review authors perform study selection in duplicate?
Q6: Did the review authors perform data extraction in duplicate?
Q7: Did the review authors provide a list of the excluded studies and justify the exclusions?*
Q8: Did the review authors describe the included studies in adequate detail?
Q9: Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?*
Q10: Did the review authors report on the sources of funding for the studies included in the review?
Q11: If meta-analysis was performed, did the review authors use appropriate methods for the statistical combination of the results?
Q12: If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?
Q13: Did the review authors account for RoB in the primary studies when interpreting/discussing the results of the review?*
Q14: Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?
Q15: If they performed quantitative synthesis, did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?*
Q16: Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?
Rating overall confidence in the results of the review:
High: No or one non-critical weakness – the systematic review provides an accurate and comprehensive summary of the results of the available studies that address the question of interest.
Moderate: More than one non-critical weakness** – the systematic review has more than one weakness, but no critical flaws; it may provide an accurate summary of the results of the available studies that were included in the review.
Low: One critical flaw with or without non-critical weaknesses – the systematic review has a critical flaw and may not provide an accurate and comprehensive summary of the available studies that address the question of interest.
Critically low: More than one critical flaw with or without non-critical weaknesses – the review has more than one critical flaw and should not be relied on to provide an accurate and comprehensive summary of the available studies.
* domains considered critical for AMSTAR II; ** multiple non-critical weaknesses may diminish confidence in the review and it may be appropriate to move the overall appraisal down from moderate to low confidence.

Only 1 systematic review addressed the effects of BF. The findings of this study suggest a significant decrease in sleep bruxism events.

Discussion

Bruxism is a topic of interest in dentistry, so there is a need to provide scientifically proven information regarding its management. Several investigations have been carried out to confirm the safety and efficacy of various kinds of treatment aimed at solving bruxism. Systematic reviews are conducted to identify, evaluate and summarize research findings, and therefore they can provide reliable information and help guide clinical decision making. However, systematic reviews are not always carried out meticulously, and the risk of implicit bias can mislead readers and induce malpractice, as evidenced in our research, where the 6 included systematic reviews that met the eligibility criteria, when evaluated with the AMSTAR 2 tool,[15] showed a low methodological quality; the critical points were not met in most of the investigations and the lack of homogeneity in terms of study design prevented us from performing any meta-analysis of the data.

Two of the included systematic reviews analyzed OAs, and reported diverse and confusing results, mainly due to the types of splints used, heterogeneous control groups and different observation periods. Manfredini et al. mention that almost all types of OAs for intermittent use are somehow effective in reducing the episodes of bruxism.[19]

However, Jokubauskas et al. indicate that although many studies support the efficacy of OA treatment for SB, the evidence is insufficient and the main role of OAs is protection against dental wear.[20] Therefore, both authors support the idea of conducting future research with longer follow-up periods. Mainieri et al. reported that treatment with MADs resulted in a reduction in the activity of the chewing muscles, the signs and symptoms of SB, and occlusal strength as well as improvement in sleep quality, but 24% of their patients had to interrupt treatment due to TMDs, muscle pain and/or discomfort.[21] In contrast, Saueressig et al. reported positive effects of MADs in the therapy of SB, without any signs or symptoms of TMDs.[22]

Another treatment for bruxism is the application of botulinum toxin, which has been referred to in a large number of studies. For example, Fernández-Núñez et al. concluded that its effectiveness was superior to any conventional treatment for SB, largely minimizing its symptoms.[8] However, Long et al. revealed that botulinum toxin had the same efficacy as a nocturnal oral splint.[23] De la Torre Canales et al. state that such disagreement is largely due to the lack of clinical protocols, the non-standardized dosage and different dilutions of the preparations among the different commercial brands used in each study.[24] Regarding adverse effects, Lee et al.[25] and Zhang et al.[26] in their reviews reported the absence of adverse effects both at the time of treatment and after botulinum toxin injection, and if they were observed, they occurred in patients who received a dose higher than the established safe dose (≤100 IU) or who had a preexisting medical condition.[25]

Studies that analyzed the effects of centrally acting drugs show very good results in the treatment of SB. It refers, among others, to clonazepam, used not only as an anticonvulsant, since its mechanism of action is also relaxation and sedation at the muscular level, mood stabilization, and relieving insomnia/anxiety.[27] In the study by Saletu et al., 21 patients diagnosed with BS received 1 mg of clonazepam per day, which showed efficacy in the polysomnographic study, with a statistically significant reduction of BS as compared to placebo.[14] However, several authors mention that it is necessary to be cautious with this type of medication, since after a period of use of 2–4 weeks, it could cause dependence.[28,29] Clonidine has an antihypertensive effect by acting as a selective agonist of the α2 receptor, which influences the sympathetic–parasympathetic balance as well as the sleep structure and motor activities during sleep.[30] It is also used to treat migraine, chronic pain, psychiatric disorders, and SB.[27] Polysomnographic records have shown that clonidine is effective in reducing SB significantly.[31]

Recently, a group of researchers have evaluated the effect of 100 mg opipramol (a single dose), which shows positive effects in reducing SB. This information could be useful for researchers who delve into this topic of great interest, taking into consideration a larger population with a control group and long-term follow-up periods.[32]

It has also been suggested that bruxism is a risk factor for developing TMDs, since an increase in the activity of the masticatory muscles could cause joint overload and myofascial pain. However, almost all studies that point to such an association used non-validated methods to diagnose bruxism, which indicates a possible diagnostic bias that could have increased the level of significance of the discussed association.[33–35] Studies such as those by Smardz et al.[36] and Wieckiewicz et al.[37] showed through their results that the relationship between SB and TMDs was not statistically significant, clearly indicating that SB is not related to TMDs, nor does it increase the risk of the appearance of any specific diagnosis of TMDs. These studies support the importance of using scientifically proven methods for making an accurate diagnosis, and thus choosing the optimal treatment.

Regarding BF, it is a subject that is still under debate, as there are studies, such as those by Lobbezoo et al.[38] and Jokubauskas et al.,[11] which show positive effects, i.e., a reduction in the episodes of bruxism in the short term; however, the authors suggest more well-designed longitudinal studies with larger samples.

In general, with our study, we were able to demonstrate that, due to the lack of scientific evidence of good methodological quality, there is still controversy and confusion about SB. A lot of misinformation has been provided about its pathophysiology in countless articles; dentists commonly relate it to peripheral factors – dental, occlusal and skeletal, believing that occlusal corrections decrease or stop this sleep activity.[39,40] Yet, Manfredini et al. in their research questioned occlusal disharmony or premature contacts as an etiological factor, concluding that the contribution of occlusion was not statistically significant in patients with and without bruxism.[4] Similarly, in a study by Ommerborn et al., a review of the functional and occlusal role was made, and no relationship was found with regard to the occlusal parameters, or skeletal or orofacial anatomy, that could explain bruxism events; on the contrary, the authors found that the role of psychology, neurotransmitters and microarousals as central factors prevailed.[41] Obviously, as in any pathology, whether an associated disorder occurs or not depends on the adaptive capacity of the person, e.g., coping with pain or stress, and genetic predisposition.[41] Therefore, SB is no longer considered to be simply related to dental occlusion factors, but it is known to have a central origin, which involves biological factors (e.g., neurochemical factors, such as dopamine and other neurotransmitters, genetics and sleep disorders), physiological factors (e.g., brain activity, muscle activity, and cardiac and respiratory functions, etc.)and psychological factors (e.g., stress sensitivity, personality traits and anxiety).[5,42–47] This has resulted in a major transformation in our understanding of BS.

Therefore, it is important to know how SB is generated and to be aware of the different stages of sleep that

a person normally goes through. Sleep ideally should last 7–8 h, starting with wakefulness, continuing with non-REM (rapid eye movement) sleep (N1, N2, N3) until the depth of sleep or REM sleep increases, and this cycle is repeated several times during the night. However, there are moments in which the individual goes to wakefulness, and these brief periods are known as micro-arousals. The term 'micro-arousal' refers to a response or a sudden change in sleep during which the individual reaches a lighter sleep, and then there is an interruption of sleep for at least 3 s, characterized by an increase in brain, autonomic, cardiac, and muscular activity, without a complete return to consciousness.[48] Taking micro-arousals as a reference, associated pathologies can be identified, such as obstructive sleep apnea (OSA), which consists in the interruption of sleep due to the lack of air in an attempt to breathe again.[49,50] Several studies have considered the possible association between SB and OSA, as SB may be a motor reflex of the central nervous system in response to a sleep arousal and OSA leads to sleep arousals.[51–53] Another pathology associated with bruxism is gastroesophageal reflux (GER), which occurs when gastric acid passes from the stomach into the esophagus, and once it exceeds the adaptive capacity of the epithelium, it generates symptoms or histological damage.[54] Li et al. showed a strong association between bruxism and symptomatic GER, and recommended that the GER status be taken into consideration as a fundamental part of the diagnosis of bruxism.[55]

Previous studies identified that also hypoxia,[56] increased body mass index (BMI),[57] hypertension,[1,57,58] excessive daytime sleepiness[59] and snoring[60] acted as independent risk factors for SB; in this study we did not investigate these issues, and another type of study could be conducted in the future to clarify them.

Taking into account all the background described above, researchers saw the need to update information about SB, and it is in the 2018 consensus where SB is defined as the activity of the masticatory muscles during sleep (characterized as rhythmic or non-rhythmic) and where it is considered as a behavior.[3] According to its clinical consequences, it was classified as: (i) a risk factor if it presents one or more negative consequences for oral health, such as severe masticatory muscle pain or joint pain, increasing the likelihood of developing a disease, without necessarily inducing it; (ii) a protective factor if it provides one or more positive health outcomes, as in the case of OSA, where the upper airway can be prevented from collapsing or its patency can be restored during sleep, or in the case of GER, where increased salivation reduces the risk of harmful chemical tooth wear; or (iii) it is neither a risk factor or a protective factor, i.e., it is only considered a motor activity of multifactorial etiology, with no consequences at the level of oral health; it is extremely important not to overdiagnose it as a pathology, since its intensity or frequency does not mean pathogenicity.[61–64]

Conclusions

Taking the limitations of the present study into consideration, the following conclusions can be drawn: the methodological quality of the studies included in this research, after being analyzed with AMSTAR 2, proved critically low; the authors recommend future research studies of better methodological quality so that the results can be applied in clinical practice. The use of centrally acting drugs, such as clonazepam and clonidine, demonstrated efficacy in reducing the episodes of SB; however, caution is recommended in their administration because of adverse effects. Treatment with botulinum toxin, OAs and BF did not demonstrate short-term efficacy; therefore, prospective studies with a longer duration are recommended. The difficulty involved in treating SB is that it does not always need to be controlled, as in most cases, it should not be considered a pathology according to the current literature. It can be a risk factor or a protective factor; therefore, treatment should not be focused on SB as such, but on investigating the pathologies, comorbidities or associated factors that lead to its onset.

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

Lissette Cerón https://orcid.org/0000-0002-7350-2939
Mishelle Pacheco https://orcid.org/0000-0002-0457-4349
Andrés Delgado Gaete https://orcid.org/0000-0001-5586-2829
Wilson Bravo Torres https://orcid.org/0000-0002-9431-1808
Daniela Astudillo Rubio https://orcid.org/0000-0002-8154-0492

References

1. Lobbezoo F, Ahlberg J, Glaros AG, et al. Bruxism defined and graded: An international consensus. *J Oral Rehabil*. 2013;40(1):2–4. doi:10.1111/joor.12011
2. Sateia MJ. International classification of sleep disorders – third edition: Highlights and modifications. *Chest*. 2014;146(5):1387–1394. doi:10.1378/chest.14-0970
3. 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
4. Manfredini D, Visscher CM, Guarda-Nardini L, Lobbezoo F. Occlusal factors are not related to self-reported bruxism. *J Orofac Pain*. 2012;26(3):163–167. PMID:22838000.
5. Klasser GD, Rei N, Lavigne GJ. Sleep bruxism etiology: The evolution of a changing paradigm. *J Can Dent Assoc*. 2015;81:f2. PMID:25633110.

6. Patel J, Cardoso JA, Mehta S. A systematic review of botulinum toxin in the management of patients with temporomandibular disorders and bruxism. *Br Dent J*. 2019;226(9):667–672. doi:10.1038/s41415-019-0257-z
7. Sendra LA, Montez C, Vianna KC, Barboza EP. Clinical outcomes of botulinum toxin type A injections in the management of primary bruxism in adults: A systematic review. *J Prosthet Dent*. 2021;126(1):33–40. doi:10.1016/j.prosdent.2020.06.002
8. Fernández-Núñez T, Amghar-Maach S, Gay-Escoda C. Efficacy of botulinum toxin in the treatment of bruxism: Systematic review. *Med Oral Patol Oral Cir Bucal*. 2019;24(4):e416–e424. doi:10.4317/medoral.22923
9. Riley P, Glenny AM, Worthington HV, et al. Oral splints for patients with temporomandibular disorders or bruxism: A systematic review and economic evaluation. *Health Technol Assess*. 2020;24(7):1–224. doi:10.3310/hta24070
10. Wang LF, Long H, Deng M, et al. Biofeedback treatment for sleep bruxism: A systematic review. *Sleep Breath*. 2014;18(2):235–242. doi:10.1007/s11325-013-0871-y
11. Jokubauskas L, Baltrušaitytė A. Efficacy of biofeedback therapy on sleep bruxism: A systematic review and meta-analysis. *J Oral Rehabil*. 2018;45(6):485–495. doi:10.1111/joor.12628
12. de Paula Gomes CA, El Hage Y, Amaral AP, Politti F, Biasotto-Gonzalez DA. Effects of massage therapy and occlusal splint therapy on electromyographic activity and the intensity of signs and symptoms in individuals with temporomandibular disorder and sleep bruxism: A randomized clinical trial. *Chiropr Man Ther*. 2014;22(1):43. doi:10.1186/s12998-014-0043-6
13. Amorim CS, Espirito Santo AS, Sommer M, Marques AP. Effect of physical therapy in bruxism treatment: A systematic review. *J Manipulative Physiol Ther*. 2018;41(5):389–404. doi:10.1016/j.jmpt.2017.10.014
14. Saletu A, Parapatics S, Anderer P, Matejka M, Saletu B. Controlled clinical, polysomnographic and psychometric studies on differences between sleep bruxers and controls and acute effects of clonazepam as compared with placebo. *Eur Arch Psychiatry Clin Neurosci*. 2010;260(2):163–174. doi:10.1007/s00406-009-0034-0
15. Shea BJ, Reeves BC, Wells G, et al. AMSTAR 2: A critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. *BMJ*. 2017;358:j4008. doi:10.1136/bmj.j4008
16. Page MJ, McKenzie JE, Bossuyt PM. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *Rev Esp Cardiol*. 2021;74(9):790–799. doi:10.1016/j.recesp.2021.06.016
17. International Platform of Registered Systematic Review and Meta-analysis Protocols (INPLASY). INPLASY's guidance for registering systematic review protocols (2021). https://inplasy.com/. Accessed December 10, 2021.
18. Ågren M, Sahin C, Pettersson M. The effect of botulinum toxin injections on bruxism: A systematic review. *J Oral Rehabil*. 2020;47(3):395–402. doi:10.1111/joor.12914
19. Manfredini D, Ahlberg J, Winocur E, Lobbezoo F. Management of sleep bruxism in adults: A qualitative systematic literature review. *J Oral Rehabil*. 2015;42(11):862–874. doi:10.1111/joor.12322
20. Jokubauskas L, Baltrušaitytė A, Pileičikienė G. Oral appliances for managing sleep bruxism in adults: A systematic review from 2007 to 2017. *J Oral Rehabil*. 2018;45(1):81–95. doi:10.1111/joor.12558
21. Mainieri VC, Saueressig AC, Fagondes SC, Teixeira ER, Seitenfus Rehm DD, Grossi ML. Analysis of the effects of a mandibular advancement device on sleep bruxism using polysomnography, the BiteStrip, the sleep assessment questionnaire, and occlusal force. *Int J Prosthodont*. 2014;27(2):119–126. doi:10.11607/ijp.3675
22. Saueressig AC, Mainieri VC, Grossi PK, et al. Analysis of the influence of a mandibular advancement device on sleep and sleep bruxism scores by means of the BiteStrip and the Sleep Assessment Questionnaire. *Int J Prosthodont*. 2010;23(3):204–213. PMID:20552084.
23. Long H, Liao Z, Wang Y, Liao L, Lai W. Efficacy of botulinum toxins on bruxism: An evidence-based review. *Int Dent J*. 2012;62(1):1–5. doi:10.1111/j.1875-595X.2011.00085.x
24. De la Torre Canales G, Câmara-Souza MB, do Amaral CF, Rodrigues Garcia RC, Manfredini D. Is there enough evidence to use botulinum toxin injections for bruxism management? A systematic literature review. *Clin Oral Investig*. 2017;21(3):727–734. doi:10.1007/s00784-017-2092-4
25. Lee SJ, McCall WD Jr., Kim YK, Chung SC, Chung JW. Effect of botulinum toxin injection on nocturnal bruxism: A randomized controlled trial. *Am J Phys Med Rehabil*. 2010;89(1):16–23. doi:10.1097/PHM.0b013e3181bc0c78
26. Zhang LD, Liu Q, Zou DR, Yu LF. Occlusal force characteristics of masseteric muscles after intramuscular injection of botulinum toxin A (BTX-A) for treatment of temporomandibular disorder. *Br J Oral Maxillofac Surg*. 2016;54(7):736–740. doi:10.1016/j.bjoms.2016.04.008
27. de Baat C, Verhoeff M, Ahlberg J, et al. Medications and addictive substances potentially inducing or attenuating sleep bruxism and/or awake bruxism. *J Oral Rehabil*. 2021;48(3):343–354. doi:10.1111/joor.13061
28. Quagliato LA, Freire RC, Nardi AE. Risks and benefits of medications for panic disorder: A comparison of SSRIs and benzodiazepines. *Expert Opin Drug Saf*. 2018;17(3):315–324. doi:10.1080/14740338.2018.1429403
29. Soyata AZ, Oflaz S. Gabapentin treatment in bruxism associated with fluoxetine. *J Clin Psychopharmacol*. 2015;35(4):481–483. doi:10.1097/JCP.0000000000000337
30. Carra MC, Macaluso GM, Rompré PH, et al. Clonidine has a paradoxical effect on cyclic arousal and sleep bruxism during NREM sleep. *Sleep*. 2010;33(12):1711–1716. doi:10.1093/sleep/33.12.1711
31. Sakai T, Kato T, Yoshizawa S, et al. Effect of clonazepam and clonidine on primary sleep bruxism: A double-blind, crossover, placebo-controlled trial. *J Sleep Res*. 2017;26(1):73–83. doi:10.1111/jsr.12442
32. Wieckiewicz M, Martynowicz H, Wieczorek T, et al. Consecutive controlled case series on effectiveness of opipramol in severe sleep bruxism management – preliminary study on new therapeutic path. *Brain Sci*. 2021;11(2):146. doi:10.3390/brainsci11020146
33. Huhtela OS, Näpänkangas R, Joensuu T, Raustia A, Kunttu K, Sipilä K. Self-reported bruxism and symptoms of temporomandibular disorders in Finnish university students. *J Oral Facial Pain Headache*. 2016;30(4):311–317. doi:10.11607/ofph.1674
34. Aguilera AB, Lopez LG, Aguilera EB, et al. Relationship between self-reported sleep bruxism and pain in patients with temporomandibular disorders. *J Oral Rehabil*. 2014;41(8):564–572. doi:10.1111/joor.12172
35. Sierwald I, John MT, Schierz O, et al. Association of temporomandibular disorder pain with awake and sleep bruxism in adults. *J Orofac Orthop*. 2015;76(4):305–317. doi:10.1007/s00056-015-0293-5
36. Smardz J, Martynowicz H, Michalek-Zrabkowska M, et al. Sleep bruxism and occurrence of temporomandibular disorders-related pain: A polysomnographic study. *Front Neurol*. 2019;10:168. doi:10.3389/fneur.2019.00168
37. Wieckiewicz M, Smardz J, Martynowicz H, Wojakowska A, Mazur G, Winocur E. Distribution of temporomandibular disorders among sleep bruxers and non-bruxers – a polysomnographic study. *J Oral Rehabil*. 2020;47(7):820–826. doi:10.1111/joor.12955
38. Lobbezoo F, Ahlberg J, Glaros AG, et al. Bruxism defined and graded: An international consensus. *J Oral Rehabil*. 2013;40(1):2–4. doi:10.1111/joor.12011
39. García DN, Cabrera LG, Reyes OR, Méndez DN. Tendencias contemporáneas de las bases fisiopatológicas del bruxismo. *Medisan*. 2014;18(8):1149–1156. https://doaj.org/article/03c406924a014bd6b0602c850ab3c827. Accessed December 10, 2021.
40. Reyes BH, Díaz Gómez SM, Hidalgo SH, Nodarse RL. Bruxismo: panorámica actual. *Arch Méd Camagüey*. 2017;21(1):913–930. http://revistaamc.sld.cu/index.php/amc/article/view/4817/2664. Accessed December 10, 2021.
41. Ommerborn MA, Giraki M, Schneider C, et al. Effects of sleep bruxism on functional and occlusal parameters: A prospective controlled investigation. *Int J Oral Sci*. 2012;4(3):141–145. doi:10.1038/ijos.2012.48
42. Lobbezoo F, Naeije M. Bruxism is mainly regulated centrally, not peripherally. *J Oral Rehabil*. 2001;28(12):1085–1091. doi:10.1046/j.1365-2842.2001.00839.x
43. Oyarzo JF, Valdés C, Bravo R. Etiología, diagnóstico y manejo de bruxismo de sueño. *Rev Méd Clín Las Condes*. 2021;32(5):603–610. https://doaj.org/article/0dbed79825e64eb19efa2c5e191b6438. Accessed December 10, 2021.
44. Carra MC, Huynh N, Lavigne G. Sleep bruxism: A comprehensive overview for the dental clinician interested in sleep medicine. *Dent Clin North Am*. 2012;56(2):387–413. doi:10.1016/j.cden.2012.01.003
45. Koyano K, Tsukiyama Y, Ichiki R, Kuwata T. Assessment of bruxism in the clinic. *J Oral Rehabil*. 2008;35(7):495–508. doi:10.1111/j.1365-2842.2008.01880.x

46. Manfredini D, Serra-Negra J, Carboncini F, Lobbezoo F. Current concepts of bruxism. *Int J Prosthodont*. 2017;30(5):437–438. doi:10.11607/ijp.5210
47. Kuhn M, Türp JC. Risk factors for bruxism. *Swiss Dent J*. 2018;128(2):118–124. PMID:29533049.
48. McNamara P, Johnson P, McLaren D, Harris E, Beauharnais C, Auerbach S. REM and NREM sleep mentation. *Int Rev Neurobiol*. 2010;92:69–86. doi:10.1016/S0074-7742(10)92004-7
49. Maspero C, Giannini L, Galbiati G, Rosso G, Farronato G. Obstructive sleep apnea syndrome: A literature review. *Minerva Stomatol*. 2015;64(2):97–109. PMID:25747430.
50. Patel SR. Obstructive sleep apnea. *Ann Intern Med*. 2019;171(11):ITC81–ITC96. doi:10.7326/AITC201912030
51. Tachibana M, Kato T, Kato-Nishimura K, Matsuzawa S, Mohri I, Taniike M. Associations of sleep bruxism with age, sleep apnea, and daytime problematic behaviors in children. *Oral Dis*. 2016;22(6):557–565. doi:10.1111/odi.12492
52. Hosoya H, Kitaura H, Hashimoto T, et al. Relationship between sleep bruxism and sleep respiratory events in patients with obstructive sleep apnea syndrome. *Sleep Breath*. 2014;18(4):837–844. doi:10.1007/s11325-014-0953-5
53. da Costa Lopes AJ, Abrahão Cunha TC, Magalhães Monteiro MC, Serra-Negra JM, Cabral LC, Simamoto PC Jr. Is there an association between sleep bruxism and obstructive sleep apnea syndrome? A systematic review. *Sleep Breath*. 2020;24(3):913–921. doi:10.1007/s11325-019-01919-y
54. Ohmure H, Oikawa K, Kanematsu K, et al. Influence of experimental esophageal acidification on sleep bruxism: A randomized trial. *J Dent Res*. 2011;90(5):665–671. doi:10.1177/0022034510393516
55. Li Y, Yu F, Niu L, Long Y, Tay FR, Chen J. Association between bruxism and symptomatic gastroesophageal reflux disease: A case–control study. *J Dent*. 2018;77:51–58. doi:10.1016/j.jdent.2018.07.005
56. Dumais IE, Lavigne GJ, Carra MC, Rompré PH, Huynh NT. Could transient hypoxia be associated with rhythmic masticatory muscle activity in sleep bruxism in the absence of sleep-disordered breathing? A preliminary report. *J Oral Rehabil*. 2015;42(11):810–818. doi:10.1111/joor.12323
57. Martynowicz H, Dymczyk P, Dominiak M, et al. Evaluation of intensity of sleep bruxism in arterial hypertension. *J Clin Med*. 2018;7(10):327. doi:10.3390/jcm7100327
58. Nashed A, Lanfranchi P, Rompré P, et al. Sleep bruxism is associated with a rise in arterial blood pressure. *Sleep*. 2012;35(4):529–536. doi:10.5665/sleep.1740
59. Câmara-Souza MB, Costa de Figueredo OM, Rodrigues Garcia RC. Association of sleep bruxism with oral health-related quality of life and sleep quality. *Clin Oral Investig*. 2019;23(1):245–251. doi:10.1007/s00784-018-2431-0
60. Michalek-Zrabkowska M, Wieckiewicz M, Macek P, et al. The relationship between simple snoring and sleep bruxism: A polysomnographic study. *Int J Environ Res Public Health*. 2020;17(23):8960. doi:10.3390/ijerph17238960
61. Polmann H, Réus JC, Massignan C, et al. Association between sleep bruxism and stress symptoms in adults: A systematic review and meta-analysis. *J Oral Rehabil*. 2021;48(5):621–631. doi:10.1111/joor.13142
62. Polmann H, Domingos FL, Melo G, et al. Association between sleep bruxism and anxiety symptoms in adults: A systematic review. *J Oral Rehabil*. 2019;46(5):482–491. doi:10.1111/joor.12785
63. Bertazzo-Silveira E, Kruger CM, De Toledo IP, et al. Association between sleep bruxism and alcohol, caffeine, tobacco, and drug abuse: A systematic review. *J Am Dent Assoc*. 2016;147(11):859–866.e4. doi:10.1016/j.adaj.2016.06.014
64. Melo G, Dutra KL, Filho RR, et al. Association between psychotropic medications and presence of sleep bruxism: A systematic review. *J Oral Rehabil*. 2018;45(7):545–554. doi:10.1111/joor.12633

Review

Survival rate and clinico-radiographic parameters around narrow-diameter dental implants for fixed dental prostheses in the posterior regions: A systematic review

Ibrahim F. Alshiddi[A–F]

Department of Prosthetic Dental Science, College of Dentistry, King Saud University, Riyadh, 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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):345–353

Address for correspondence
Ibrahim F. Alshiddi
E-mail: shidiibra147@gmail.co

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on March 18, 2021
Reviewed on July 3, 2021
Accepted on August 2, 2021

Published online on June 30, 2023

Abstract

Narrow-diameter dental implants (NDDIs) are suggested to be a reliable alternative to bone augmentation techniques, but evidence regarding the feasibility of NDDIs in the posterior area is limited. This review investigated the survival rates of NDDIs, as well as peri-implant clinical and radiographic parameters for a fixed dental prosthesis in posterior regions compared to standard-diameter dental implants (SDDIs). One investigator performed an electronic search of the English literature in the Web of Science, PubMed, Scopus, and EMBASE databases until December 2020. The focused question was: "Do narrow diameter dental implants restoring fixed dental prosthesis demonstrate more alveolar bone loss as compared to standard diameter dental implants in posterior maxillary and mandibular regions?"

The 9 studies selected for this review assessed a total of 498 patients (250 males, 206 females, aged 19–86 years), 725 NDDIs and 260 SDDIs were placed. The mean follow-up duration was 71 months (range: 12–176 months). A high survival rate of NDDIs was noticed (i.e., 97.37% [range: 94.7–100%]). The mean probing depth and bleeding on probing scores ranged between 3.67 mm and 3.12 mm, and 10% and 33.42%, respectively. However, the only study reporting plaque index (PI) demonstrated a mean PI score of 1.39. The majority of the studies reported mean marginal bone loss scores below 1.0 mm.

In conclusion, NDDIs appear to be a feasible treatment option in patients requiring fixed dental prosthesis in posterior regions since they exhibit comparable survival rates to SDDIs, as well as a clinically acceptable peri-implant clinical and radiographic tissue response.

Keywords: marginal bone loss, diameter, narrow diameter, survival, systematic review

Cite as
Alshiddi IF. Survival rate and clinico-radiographic parameters around narrow-diameter dental implants for fixed dental prostheses in the posterior regions: A systematic review. *Dent Med Probl.* 2023;60(2):345–353. doi:10.17219/dmp/140757

DOI
10.17219/dmp/140757

Introduction

Excellent predictability and high success rates of dental implant therapy have been revealed by countless clinical reports.[1–3] Additionally, rehabilitation of the oral cavity utilizing dental implants may yield an improved oral health-related quality of life.[4,5] However, atrophy of the alveolar crest with decreased bone height and width because of marginal periodontitis, denture wearing, neoplasia, malformation, and trauma makes the placement of dental implants challenging. In such scenarios, supplementary surgical protocols can be needed to increase the inadequate alveolar bone volume and reconstruct the harmful sagittal, horizontal, or vertical inter-maxillary relationships.[6] In this aspect, numerous augmentation methods are explained in the literature, based on the size and site of the defect, including lateral and/or vertical alveolar ridge augmentation and maxillary sinus floor augmentation.[6] However, these augmentation methods are costly and time-consuming and require surgical skills to minimize patient morbidity and prevent several complications, including wound dehiscence, hemorrhage, bone fractures, nerve damage, infections, postoperative pain, and augmentation or dental implant failure.[7,8] Moreover, augmentation methods might carry an increased risk of complications in medically compromised patients (i.e., patients utilizing anti-resorptive drugs or having a history of radiation to the oral and maxillofacial region).[8]

Implant survival depends on the maintenance of optimal conditions of peri-implant tissues to achieve stability. After obtaining osseointegration, the failure of the implant is linked to the gradual loss of peri-implant bone tissue, which is due to inflammation in the area.[9] Therefore, it is essential to monitor peri-implant bone levels at regular follow-ups after the implant treatment is completed. If no adequate oral hygiene instructions are being followed or the implant is overloaded, it may cause peri-implantitis and loss of the implant.[10]

Narrow-diameter dental implants (NDDIs) are becoming a growing scientific and clinical interest. By utilizing NDDIs, patient morbidity may be decreased by avoiding augmentation or other invasive surgical procedures.[11] Until recently, the application of NDDIs has been limited to the replacement of teeth having narrow clinical crowns and/or for limited inter-implant or inter-dental spaces, including mandibular incisors and maxillary lateral regions.[12] Several studies have reported the survival rate of NDDIs being comparable to standard diameter dental implants (SDDIs).[13,14] It might be postulated that dental implant survival is not associated with implant diameter. However, the utilization of NDDIs is avoided in the posterior regions due to biomechanical and prosthetic considerations.[12]

Some studies comparing NDDIs with SDDIs have reported comparable dental implant survival rates for both implant options.[15–16] However, studies have suggested a few limitations with the utilization of NDDIs that should be taken into account, including considerably lower fracture resistance in comparison to SDDIs, which increases the risk of dental implant failure.[17–20] According to Ivanoff and colleagues[21], a decrease in implant diameter results in a decreased implant-to-bone contact region, yielding a lower surface area, hence the small diameter may undermine the osseointegration of NDDIs. However, recent studies have demonstrated that NDDIs might be employed for the replacement of posterior and anterior teeth, with comparable clinical and radiographic results.[21,22]

Till today, the application of NDDIs has been limited to specifically defined indications having relatively low occlusal loading, such as incisors or to retain elements for overdentures. Before NDDIs can be recommended in a wider clinical setup, the assessment of available evidence is essential.[23] Since there is no clinical evidence regarding peri-implant clinical and radiographic parameters around NDDIs for fixed dental prostheses in the posterior region in comparison to SDDIs. Therefore, this systematic review aimed to investigate the survival rates of NDDIs as well as peri-implant clinical and radiographic parameters for fixed dental prostheses in posterior maxillary and mandibular regions compared to SDDIs.

Material and methods

The current systematic review was constructed in agreement with the guidelines provided by the Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) statement.[24]

Focused question and study outcomes

The focused PICO question that was answered is as follows[25]: "Do narrow diameter dental implants (intervention) restoring a fixed dental prosthesis (patient) demonstrate more alveolar bone loss (outcome) as compared to standard diameter dental implants (comparison) in posterior maxillary and mandibular regions?"

The survival rates (i.e., time from first placement to functional loss of the dental implant) of NDDIs were the primary outcome, while peri-implant clinical (i.e., plaque index (PI), probing depth (PD), bleeding on probing (BOP)) and radiographic (marginal bone loss (MBL) parameters constituted the secondary outcomes.

Patient selection and intervention

The current review included studies of patients scheduled for placement of a minimum of 1 NDDI into the mandible and/or maxilla with a narrow alveolar ridge (i.e., inadequate bone volume). Any simultaneous bone augmentation techniques were not allowed. Studies with only healthy participants without any systemic condition

that might influence bone metabolism and the absence of local infection signs were included in the present review. No restrictions regarding the age or gender of the patients were imposed.

Only reports containing dental implants of diameters ranging from 3.2 mm to 3.5 mm were included without any restriction to dental implant length. The NDDI's indication included the restoration of a fixed dental prosthesis.

Search strategy

The authors searched the Clarivate Analytics' Web of Science (All Databases), Medline/PubMed, Elsevier's Scopus, and EMBASE databases from January 1990 to December 2020 for suitable publications in English to address the focused question. The complete search was carried out related to the articles that compared the alveolar bone loss levels after restoring a fixed dental prosthesis with NDDIs and SDDIs. The following search terms were used in all search databases: "small implant*" OR "small dental implant*" OR "small diameter implant*" OR "small diameter dental implant" OR "narrow implant*" OR "narrow dental implant*" OR "narrow diameter implant*" OR "narrow diameter dental implant*" OR "standard implant*" OR "standard dental implant*" "standard diameter implant*" OR "standard diameter dental implant*" "regular implant*" "regular dental implant*" OR "regular diameter implant*" OR "regular diameter dental implant*" OR "diameter implant*" OR "diameter dental implant*" OR "mini-implant*" OR "mini dental implant*".

The titles and abstracts for the eligible studies were screened by two authors. If the abstract did not contain data relevant to the eligibility criteria. If the title of the article was relevant, but the abstract was not available, the article was chosen for a thorough reading of the text. Then, the identification of full-text articles that fulfilled the eligibility criteria was performed. Manual searching of the clinical studies' reference lists was carried out to identify papers that could have been missed during the electronic search. The following journals were selected for manual searching: (a) *Journal of Periodontology,* (b) *Journal of Clinical Periodontology,* (c) *Journal of Periodontal Research,* (d) *Clinical Oral Implants Research,* (e) *Clinical Implant Dentistry Related Research,* (f) *European Journal of Oral Implantology,* (g) *International Journal of Implant Dentistry,* (h) *Journal of Periodontal and Implant Science,* (i) *International Journal of Periodontics and Restorative Dentistry,* (j) *Implant Dentistry,* (k) *Journal of Oral Implantology,* and (l) *Implantologie*. Articles that fulfilled the selection criteria were analyzed for data extraction.

Data extraction

The following data from the selected studies were extracted and tabulated: (a) year, author, and journal of publication, (b) methodological design of the study, (c) gender and mean age (year) of the study participants, (d) the total number of dental implants placed in the maxilla and/or mandible, (e) diameter (in mm), (f) placement depth of dental implants, (g) follow-up duration, (h) survival rate of the dental implant, and (i) outcome of the study. The information gathered was based on the focused question proposed for the current systematic review.

Eligibility criteria

The inclusion criteria included: (a) studies on the survival rates of dental implants under functional loading, (b) studies that analyzed peri-implant clinical and radiographic parameters, (c) studies having at least 12 treated patients, (d) studies with a mean follow-up duration of dental implant survival of a minimum of one-year post-implant placement, and (e) studies published in the English language. The following methodological designs were included:

– prospective: cohort, non-randomized-controlled, randomized-controlled studies;
– retrospective: single-cohort, controlled trials.

The exclusion criteria comprised of (a) studies having a mean follow-up period of < 1 year, (b) studies discussing simultaneous bone augmentation techniques, (c) the utilization of mini-dental implants for orthodontic anchorage, (d) reviews, expert opinions, case reports, case series, animal studies, or *in-vitro* studies, (e) studies with < 12 patients, and (f) studies published in languages other than English.

Risk of bias/quality assessment

Two investigators evaluated the study quality of the included studies. The quality assessment of the randomized controlled trials was carried out utilizing the revised recommendations of the Consolidated Standards of Reporting Trials (CONSORT) statement.[26] The estimation of quality assessment for each included randomized controlled trial was based on the Cochrane Handbook for Systematic Reviews of Interventions.[27] In summary, the following sections were taken into account: (a) reporting bias (selective reporting), (b) attrition bias (incomplete outcome data), (c) detection bias (blinding of outcome evaluation), (d) performance bias (blinding of research subjects), (e) selection bias (randomization and allocation concealment), and (f) other biases. For each of these sections, the reports were categorized as having a low risk of bias (low), high risk of bias (high), or unclear (?). Overall, reports were regarded as (a) high risk of bias if ≥ 1 criterion was not fulfilled, (b) low risk of bias if all criteria were fulfilled, and (c) unclear risk of bias if ≥ 1 criterion was partly fulfilled.

For assessing the quality of non-randomized and observational studies, the grading scale proposed by the Newcastle-Ottawa Scale (NOS) was used.[28] This scale utilizes

a star system to assess the reports on 3 comprehensive aspects[29]: (a) the identification of either outcome or exposure of interest (maximum three stars), (b) the comparability of study groups (maximum two stars), and (c) the selection of the research groups (maximum four stars).

Additional analysis

Due to deficient high-quality evidence, limited data presented in some studies, and the discrepancy between the methods, the utilization of advanced statistical methods was felt inappropriate. The data quality was also very limited to carrying out any statistical bias evaluations. The secondary outcomes of the present review are presented narratively.

Results

Study selection

The searches from the electronic databases (Medline/PubMed [n = 1517], Scopus [n = 346], Web of Science [n = 252], and EMBASE [n = 158]) yielded 2273 relevant publications for consideration. After the screening of titles and abstracts, 29 studies were chosen for full-text review, and 2244 studies, after the removal of duplicates, did not meet the inclusion criteria and were excluded. The screening of titles and abstracts resulted in the exclusion of 20 more studies. Overall, 9 studies were finally selected for the present systematic review (Figure 1). All studies were conducted at either private dental clinics, healthcare centers, or universities.

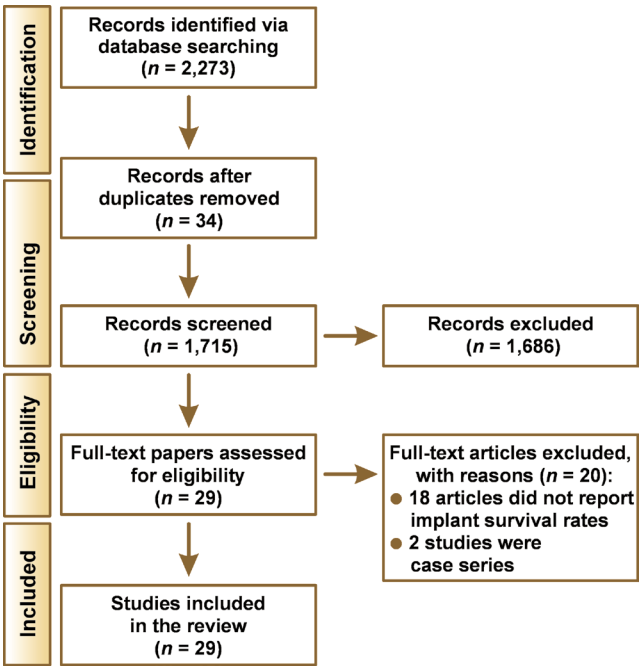

Fig. 1. PRISMA flow diagram for the literature search

General characteristics of the included studies

Table 1 depicts the general characteristics of the included studies. In the present review, four studies were retrospective clinical trials[30–33], two were prospective clinical trials[34,35], two were retrospective cohort studies[36,37], and one study was a randomized controlled trial.[38] The majority of the clinical trials were published during the 2010s, however, one study was published in 2009. These studies were carried out in Belgium, Brazil, China, Italy, Portugal, the Republic of Korea, and Turkey, and were published in the *International Journal of Implantology*, *Clinical Oral Implants Research*, *Journal of Periodontology*, *Maxillofacial Plastic and Reconstructive Surgery*, *Oral Implantology*, *Implant Dentistry*, and *Clinical Implant Dentistry Related Research*. A total of 498 participants were involved in the studies and included in the present review, with male participants (n = 250) being slightly more than female patients (n = 206). However, a study involving 42 participants did not mention the gender of the patients.[30] Overall, the range of study participant numbers was between 12 and 147, and the age range was 19–81 years. The total number of NDDIs was 725, while the total number of SDDIs was only 260. Overall, 380 implants were placed in the maxilla, whereas 360 implants were placed in the mandible. One study did not report the jaw(s) in which implants were placed.[37] The diameter of NDDIs ranged from 3.2 mm to 3.5 mm, while the diameter of SDDIs ranged from 3.5 mm to 4.1 mm. Overall, 8 studies applied bone-level dental implants, whereas only 1 study utilized tissue-level dental implants.[38]

Survival rate of NDDIs

All the included reports presented the survival rate of NDDIs. Overall, the survival rate of the NDDIs ranged from 94.7% to 100%. Out of a total of 725 NDDIs used, only 19 failed, which corresponds to an NDDI survival rate of 97.37% during the follow-up time, which ranged from 12 months to 176 months. A higher failure rate of NDDIs was observed in the maxillary arch (n = 11, [57.89%]) as compared to the mandibular arch (n = 8, [42.10%]).

Peri-implant clinical and radiographic parameters

Only one study assessed PI, PD, and BOP[36], while two studies evaluated only BOP and PD[37,38], and one study evaluated only BOP.[30] Overall, the mean PPD and BOP scores ranged between 3.67 mm and 3.12 mm, and 10% and 33.42%, respectively. However, the only study reporting PI demonstrated a mean PI score of 1.39.[36]

All the included studies reported MBL around NDDIs. The majority of the studies (n = 8) reported mean MBL scores below 1.0 mm, except one report that demonstrated mean MBL scores of 1.16 mm.[36]

Table 1. General description and implant-related characteristics of the included studies

Author; publication year; journal name; country	Study design	Participants; (male + female); age (range)	No. of implants (*n*); max/mand	Diameter (mm)	Placement depth	Follow-up (months)	Survival rate (%)	Study outcome
Eskan et al., [1]; 2020; Int J Implant Dent; Turkey	Retrospective clinical study	n=42 (-)	n: 171 NDDIs: (n=37) SDDIs: (n=134); 98/73	NDDIs: 3.3 SDDIs: 4.1	Bone level	55	100	NDDI successfully treated total edentulous patients requiring an immediate implant placement and loading.
Shi et al., [2]; 2017; Clin Oral Implants Res; China	Retrospective cohort study	n=67; (38+29); (21-56 years) 35.6 years	N: 114 NDDIs: (n=114); 42/56	NDDIs: 3.3	Bone level	96-176 (121)	97	NDDI, being a predictable treatment option, has high survival rates, high patient satisfaction, acceptable complication rates and marginal bone loss.
Pieri et al., [3]; 2016; J Periodontol; Italy	Retrospective cohort study	n=107; (33+74); (44-81 years) 61.02 years	n: 239 NDDIs: n=113 SDDIs: n=126 -/-	NDDIs: 3.3 SDDIs: 3.5-4.0	Bone level	60	99.1	Fixed partial denture treatment in posterior jaws with NDDIs was as reliable as with SDDIs, although NDDIs showed a higher risk of prosthetic complications.
Woo et al., [4]; 2016; Maxillofac Plast Reconstr Surg; Republic of Korea	Retrospective clinical study	n=66 (37+29); (19-76 years) 51.4 years	n: 98 NDDIs: (n=98); 42/56	NDDIs: 3.5	Bone level	12-48	100	NDDIs must be considered an alternative for SDDIs to restore a posterior edentulous region.
Tolentino et al., [5]; 2016; Clin Oral Implants Res; Brazil	Randomized controlled trial	n=12 (4+8); 43.3 years	n= - NDDIs: - Mandible: all	NDDIs: 3.5	Tissue level	12	100	NDDIs may be equally used to support single crowns in the posterior area of the mouth.
Lambert et al., [6]; 2015; J Oral Implantol; Belgium	Prospective clinical study	n=20 (5+15); 21-70 years	n=39 NDDIs: (n=39); -/-39	NDDIs: 3.3	Bone level	12	94.7	Use of NDDIs to restore partial edentation in sites with limited horizontal thickness seems to be an effective treatment option that prevented guided bone regeneration in the majority of the present cases.
Garcez-Filho et al., [7]; 2014; Clin Oral Implants Res; Brazil	Retrospective clinical study	n=21 (9+12); (33-78 years) 55.5 years	n: 40 NDDIs: (n=40); 40/-	NDDIs: 3.3	Bone level	120	97	NDDIs installed immediately after split-crest procedure may successfully support prosthetic rehabilitations after long-time intervals.
Mangano et al., [8]; 2013; Implant Dent; Italy	Prospective clinical study	n=16 (9+7); (48-69 years) 58.5 years	n=37 NDDIs: (n=37); 14/23	NDDIs: 3.2	Bone level	24	100	NDDIs can be used in fixed prosthetic rehabilitations in the posterior regions of both jaws with a predictable positive outcome.
Malo et al., [9]; 2009; Clin Implant Dent Relat Res; Portugal	Retrospective clinical study	n=147 (115+32); (26-77 years) 47.5 years	n=247 NDDIs: (n=247) 144/103	NDDIs: 3.3	Bone level	12-132 (60)	95.1	The use of NDDIs for the prosthetic rehabilitation of posterior regions of the jaws is viable, with good outcomes in the long-term, irrespective of the surgical technique implemented.

Abbreviations: NDDIs= narrow-diameter dental implants; SDDIs= standard-diameter dental implants.

Patient satisfaction

Out of 9 included studies, only one study evaluated patient satisfaction, using a visual analog scale.[36] Approximately 90% and 85% of the patients were satisfied with the aesthetics and function of the NDDI and SDDI restorations, respectively. However, around 11% and 15% of the patients were not satisfied with the aesthetics and function of the restorations, respectively, mainly due to food impaction and buccal mucosal recession. The overall satisfaction of the visual analog scale was 9.21 (SD: 1.53).

Study outcomes

The majority of included studies (8/9) concluded that NDDIs are a reliable and predictable method to suc-

cessfully treat patients that require an immediate dental implant treatment for restoring posterior maxillary and mandibular edentulous regions. According to one report[37], fixed partial denture therapy in the posterior region using NDDIs was a reliable option, although a higher risk of prosthetic complications was observed with the utilization of NDDIs.

Risk of bias/quality assessment

A wide variety of quality assessments was observed across the included studies during the quantitative analysis (Table 2). One study was of excellent quality[38], three studies were of good quality[34,35,37], and five studies were of moderate quality.[30–33,36]

Discussion

The present systematic review aimed to investigate the clinical and radiographic parameters around NDDIs for a fixed dental prosthesis in posterior maxillary and mandibular regions in comparison to SDDIs. There is a dearth of literature on clinical studies, particularly randomized controlled trials, regarding the survival rates of NDDIs for fixed dental prosthesis in posterior regions. Hence, the present review also included observational studies. Since the rarity of randomized controlled trials, a huge variation in the quality assessment regarding the included studies was seen. Moreover, an enormous variety was observed in survival follow-up duration. Most of the studies did not mention the reasons for implant success and implant failure. Hence, these factors have to be taken into account while interpreting the outcomes of the current review in comparison to other reviews that included randomized controlled trials only.

In the present systematic review, mini dental implants (MDIs) were not included to perform a more precise search. This explicit differentiation between NDDIs and MDIs was established to solve all possible confusion. The Glossary of Oral and Maxillofacial Implants (GOMI) definition of MDIs and threshold of an implant diameter > 3 mm was applied.[39] According to the authors of the present review, a dental implant having a diameter of > 3 mm is best considered to be an NDDI, which is considerably different from a MDI. This adoption of the GOMI definition and 3 mm implant diameter threshold is recommended for future studies to differentiate between NDDIs and MDIs.

In the present review, survival rates of NDDIs seem to be comparable to those of SDDIs (> 3.5 mm). Most of the included studies demonstrated survival rates > 97%, and no report presented survival rates below 94%. This may indicate a reliable alternative treatment option, however, assessment of the success of NDDI applications should not be performed exclusively using the identification of dental implant survival.[40] The measurement of peri-implant clinical and radiographic parameters, implant success, and indications should also be taken into account.[41] Several extrinsic and intrinsic factors might affect the stability of the peri-implant clinical and radiographic parameters. Vital intrinsic factors include the quality and quantity of surrounding soft and hard tissue. However, dental implant design, the total number of implants placed, implant angulation, and depth of insertion constitute the implant-related extrinsic factors.[40]

The survival rates of NDDIs for fixed dental prostheses could also be influenced by the higher fracture and failure risk.[20] The former is possible because of their small surface area in contact with the alveolar bone tissue in comparison with SDDIs. Because of these risks, NDDIs are preferably employed only in scenarios with reduced ridge thickness or in cases having limited space.[42–44] In the present review, an overall survival rate of around 98% was reported, comparable to what is reported in other studies related to SDDIs, i.e., 97%[45] and 99%[46]. The NDDIs survival rates reported in the present study are well above the success criterion proposed by Albrektsson and colleagues in 1986[47], which is 85% at 5-year follow-up and 80% at 10-year follow-up. The decreased pain linked to NDDIs is a supplementary advantage. However, this decreased pain perception might be more related to the decreased surgical steps since a bone grafting procedure is not necessary for placing NDDIs.[48]

Table 2. Assessment of the quality of the studies included in the systematic review

Author (year)	Selection	Comparability	Outcome	Score	Quality
Eskan et al., (2020)	***	*	*	5	Moderate
Shi et al., (2017)	**	*	*	4	Moderate
Pieri et al., (2016)	***	**	*	6	Good
Woo et al., (2016)	***	*	*	5	Moderate
Tolentino et al., (2016)	***	***	***	9	Excellent
Lambert et al., (2015)	***	**	*	6	Good
Garcez-Filho et al., (2014)	***	*	*	5	Moderate
Mangano et al., (2013)	**	**	**	6	Good
Malo et al., (2009)	**	*	*	4	Moderate

Vitamin D pleiotropism is of great interest in contemporary dentistry for clinicians who perform dental implant procedures since it contributes to bone metabolic processes and modulates the immune system.[49–51] It is assumed that the appropriate quantity of Vitamin D is positively associated with the process of osseointegration. Several reports demonstrate that this prohormone is potentially vital for the process of postsurgical tissue repair, as well as the integration of the dental implant with bone and peri-implant bone homeostasis after a dental prosthesis is placed.[52] Moreover, its function to reduce peri-implant inflammation is of particular importance.[53] At the interface between the dental prosthesis and implant, Vitamin D causes the induction of regional cells of the immune system (i.e., formation of 1-alpha-hydroxylase by monocytes).[53] Hence, the authors of this study recommend the use of appropriate concentrations of Vitamin D in such patients since Vitamin D treatment remarkably increases bone levels at the implant site, which might be a crucial factor in the long-term survival of dental implants.[54]

Another crucial factor that influences the predictability of NDDIs is the MBL over time.[20] According to Assaf et al.,[12] the predictability of NDDIs not only depends on their diameter but also on MBL, which should be within comparable limits as those reported for SDDIs. According to the findings of the present review, the majority of the included studies reported mean MBL scores below 1.0 mm. These findings are in agreement with the published literature that reports an acceptable MBL of 2 mm in the 1st year after placement of the dental implant, followed by 0.2 mm per annum.[55,56]

Implant type, primarily the surface macrostructure and microstructure, appears to influence the survival rate of dental implants and implant restoration designs.[12] Maló and de Araújo Nobre[33] highlighted these two factors and reported more failures using screw-shaped dental implants having smooth surfaces compared to taper-shaped and surface-treated dental implants. They also determined a potential predisposing factor for dental implant failure with partial rehabilitation in comparison with complete edentulous and single-tooth rehabilitation. However, their report had some inherent limitations: varying loading techniques, including immediate loading, were utilized, as well as varying dental implant lengths (from 10–15 mm).[33]

A decrease in diameter means a decrease in the implant-to-bone contact surface.[12] According to reports, wider diameter implants exhibit greater pull-out forces and removal torques. This explains the fact why clinicians choose an SDDI when sufficient width is available since the outcomes are in favor of SDDIs in regards to mechanical strength, initial stability, and the available surface of osseointegration.[21,57]

Patient-centered results are mostly overlooked, despite the obvious consequences on the success of dental implant treatment.[58] The majority of the included studies (8/9) did not report patient-centered results (i.e., patient satisfaction). Patient satisfaction and restoration of aesthetics and functions are the primary goals when treating the edentulous patient utilizing dental implants, and hence new reports should evaluate these vital parameters of dental implant therapy.[59] In this context, the establishment of a well-defined success benchmark is necessary for reporting and evaluating prosthetic, dental implant, and patient-centered results along with technical and biological complications.

Limitations

It is imperative to recognize the limitations of the present review. Though there has been a significant increase in the number of reports that have examined the behavior of NDDIs as compared to SDDIs for fixed dental prostheses in posterior regions, however, there are a very limited amount of randomized controlled/clinical trials. Furthermore, the difficulty of blinding the study investigators, subjects, and outcome assessors may be considered a bias of this systematic review. Hence, caution should be taken while interpreting the outcomes of this study due to a lower number of randomized controlled/clinical trials, and further clinical trials should be conducted to better answer the question as to fixed prosthodontics treatment using NDDIs in posterior regions. Despite this, the application of NDDIs for fixed prosthodontics therapy in posterior regions demonstrates high survival rates and clinically favorable peri-implant clinical and radiographic parameters.

Conclusions

Within the limitations of the present review, NDDIs appear to be a feasible treatment option in patients requiring fixed dental prosthesis in posterior regions since they exhibit comparable survival rates with SDDIs as well as clinically acceptable peri-implant clinical and radiographic tissue responses.

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

Ibrahim F. Alshiddi https://orcid.org/0000-0002-1272-604X

References

1. Al-Nawas B, Kämmerer PW, Morbach T, Ladwein C, Wegener J, Wagner W. Ten-year retrospective follow-up study of the TiOblast™ dental implant. *Clin Implant Dent Related Res*. 2012;14(1):127-134.
2. Moraschini da C. Poubel LA, Ferreira VF, dos Barboza E. Evaluation of survival and success rates of dental implants reported in longitudinal studies with a follow-up period of at least 10 years: A systematic review. *Int J Oral Maxillofac Surg*. 2015;44(3):377-388.
3. Schiegnitz E, Al-Nawas B, Tegner A, Sagheb K, Berres M, Kämmerer PW, Wagner W. Clinical and radiological long-term outcome of a tapered implant system with special emphasis on the influence of augmentation procedures. *Clin Implant Dent Related Res*. 2016;18(4):810-820.
4. Heydecke G, Locker D, Awad MA, Lund JP, Feine JS. Oral and general health-related quality of life with conventional and implant dentures. *Commun Dent Oral Epidemiol*. 2003;31(3):161-168.
5. Schiegnitz E, Kämmerer P, Sagheb K, Wendt A, Pabst A, Al-Nawas B, Klein M. Impact of maxillary sinus augmentation on oral health-related quality of life. *Int J Implant Dent*. 2017;3(1):1-8.
6. Al-Nawas B, Schiegnitz E. Augmentation procedures using bone substitute materials or autogenous bone-a systematic review and meta-analysis. *Eur J Oral Implantol*. 2014;7(Suppl 2):S219-S34.
7. Schiegnitz E, Al-Nawas B, Kämmerer P, Grötz K. Oral rehabilitation with dental implants in irradiated patients: a meta-analysis on implant survival. *Clin Oral Investig*. 2014;18(3):687-698.
8. Walter C, Al-Nawas B, Wolff T, Schiegnitz E, Grötz KA. Dental implants in patients treated with antiresorptive medication–a systematic literature review. *Int J Implant Dent*. 2016;2(1):1-15.
9. Lindhe J, Meyle J, Group D of the EuropeanWorkshop on Periodontology. Peri-implant diseases: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol*. 2008;35:282–285.
10. Mahato N, Wu X, Wang L. Management of peri-implantitis: A systematic review, 2010–2015. SpringerPlus. 2016;5:105.
11. Schiegnitz E, Al-Nawas B. Narrow-diameter implants: A systematic review and meta-analysis. *Clin Oral Implants Res*. 2018;29(Suppl 16):21-40.
12. Assaf A, Saad M, Daas M, Abdallah J, Abdallah R. Use of narrow-diameter implants in the posterior jaw: a systematic review. *Implant Dent*. 2015;24(3):294-306.
13. Cruz RS, Lemos CA, de Batista VE, Yogui FC, Oliveira HF, Verri FR. Narrow-diameter implants versus regular-diameter implants for rehabilitation of the anterior region: a systematic review and meta-analysis. *Int J Oral Maxillofac Surg*. 2020;50(5):674-682.
14. Gonzales-Valls G, Roca-Millan E, Cespedes-Sanchez JM, Gonzales-Navarro B, Torrejon-Moya A, Lopez-Lopez J. Narrow diameter dental implants as an alternative treatment for atrophoc ridges: systematic review and meta-analysis. *Materials*. 2021;14(12):3234.
15. de Souza AB, Sukekava F, Tolentino L, César-Neto J.B, Garcez-Filho J, Araújo MG. Narrow-and regular-diameter implants in the posterior region of the jaws to support single crowns: A 3-year split-mouth randomized clinical trial. *Clin Oral Implants Res*. 2018;29(1):100-107.
16. Al-Shibani N, Al-Aali KA, Al-Hamdan RS, Alrabiah M, Basunbul G, Abduljabbar T. Comparison of clinical peri-implant indices and crestal bone levels around narrow and regular diameter implants placed in diabetic and non-diabetic patients: A 3-year follow-up study. *Clin Implant Dent Related Res*. 2019;21(2):247-252.
17. Corcuera-Flores JR, Pérez-Fierro M, Blanco-Carrión A, Torres-Lagares D, Castellanos-Cosano L, Machuca-Portillo G. Bone loss around narrow implants versus standard diameter implants: Retrospective 2-years case-control study. *J Clin Exp Dent*. 2020;12(1):e79-e84.
18. Buser D, Von Arx T. Surgical procedures in partially edentulous patients with ITI implants. *Clin Oral Implants Res*. 2000;11(Suppl 1):83-100.
19. Akça K, Cehreli MC, İplikçioğlu H. Evaluation of the mechanical characteristics of the implant–abutment complex of a reduced-diameter morse-taper implant: A nonlinear finite element stress analysis. *Clin Oral Implants Res*. 2003;14(4):444-454.
20. Marcello-Machado R, Faot F, Schuster A, Nascimento G, Del Bel Cury A. Mini-implants and narrow diameter implants as mandibular overdenture retainers: A systematic review and meta-analysis of clinical and radiographic outcomes. *J Oral Rehabil*. 2018;45(2):161-183.
21. Ivanoff CJ, Sennerby L, Johansson C, Rangert B, Lekholm U. Influence of implant diameters on the integration of screw implants: an experimental study in rabbits. *Int J Oral Maxillofac Surg*. 1997;26(2):141-148.
22. Arısan V, Bölükbaşı N, Ersanlı S, Özdemir T. Evaluation of 316 narrow diameter implants followed for 5–10 years: a clinical and radiographic retrospective study. *Clin Oral Implants Res*. 2010;21(3):296-307.
23. Barter S, Stone P, Brägger U. A pilot study to evaluate the success and survival rate of titanium–zirconium implants in partially edentulous patients: results after 24 months of follow-up. *Clin Oral Implants Res*. 2012;23(7):873-881.
24. Moher D, Schulz KF, Simera I, Altman DG. Guidance for developers of health research reporting guidelines. *PLoS Med*. 2010;7(2):e1000217.
25. Stone P. Popping the (PICO) question in research and evidence-based practice. *Nurs Res*. 2002;15(3):197-198.
26. Pandis N, Chung B, Scherer RW, Elbourne D, Altman DG. CONSORT 2010 statement: extension checklist for reporting within person randomised trials. *BMJ*. 2017;357:j2835.
27. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg*. 2010;8(5):336-341.
28. Wells GA, Shea B, O'Connell DA, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Oxford; 2000.
29. Margulis AV, Pladevall M, Riera-Guardia N, Varas-Lorenzo C, Hazell L, Berkman N.D, Viswanathan M, Perez-Gutthann S. Quality assessment of observational studies in a drug-safety systematic review, comparison of two tools: the Newcastle–Ottawa scale and the RTI item bank. *Clin Epidemiol*. 2014;6:359-368.
30. Eskan MA, Uzel G, Yilmaz S. A fixed reconstruction of fully edentulous patients with immediate function using an apically tapered implant design: a retrospective clinical study. *Int J Implant Dent*. 2020;6(1):1-10.
31. Woo IH, Kim JW, Kang SY, Kim YH, Yang BE. Narrow-diameter implants with conical connection for restoring the posterior edentulous region. *Maxillofac Plast Reconstr Surg*. 2016;38(1):1-7.
32. Garcez-Filho J, Tolentino L, Sukekava F, Seabra M, Cesar-Neto J, Araújo M. Long-term outcomes from implants installed by using split-crest technique in posterior maxillae: 10 years of follow-up. *Clin Oral Implants Res*. 2015;26(3):326-331.
33. Maló P, de Araújo Nobre M. Implants (3.3 mm diameter) for the rehabilitation of edentulous posterior regions: a retrospective clinical study with up to 11 years of follow-up. *Clin Implant Dent Related Res*. 2011;13(2):95-103.
34. Lambert FE, Lecloux G, Grenade C, Bouhy A, Lamy M, Rompen EH. Less invasive surgical procedures using narrow-diameter implants: a prospective study in 20 consecutive patients. *J Oral Implantol*. 2015;41(6):693-699.
35. Mangano F, Pozzi-Taubert S, Zecca PA, Luongo G, Sammons R.L, Mangano C. Immediate restoration of fixed partial prostheses supported by one-piece narrow-diameter selective laser sintering implants: a 2-year prospective study in the posterior jaws of 16 patients. *Implant Dent*. 2013;22(4):388-393.
36. Shi JY, Xu FY, Zhuang LF, Gu YX, Qiao SC, Lai HC. Long-term outcomes of narrow diameter implants in posterior jaws: A retrospective study with at least 8-year follow-up. *Clin Oral Implants Res*. 2018;29(1):76-81.
37. Pieri F, Forlivesi C, Caselli E, Corinaldesi G. Narrow-(3.0 mm) versus standard-diameter (4.0 and 4.5 mm) implants for splinted partial fixed restoration of posterior mandibular and maxillary jaws: A 5-year retrospective cohort study. *J Periodontol*. 2017;88(4):338-347.
38. Tolentino L, Sukekava F, Garcez-Filho J, Tormena M, Lima L, Araújo M. One-year follow-up of titanium/zirconium alloy X commercially pure titanium narrow-diameter implants placed in the molar region of the mandible: a randomized controlled trial. *Clin Oral Implants Res*. 2016;27(4):393-398.
39. Laney WR. Glossary of Oral and Maxillofacial Implants. *Int J Oral Maxillofac Implants*. 2017;32:Gi-200.
40. Klein MO, Schiegnitz E, Al-Nawas B. Systematic review on success of narrow-diameter dental implants. *Int J Oral Maxillofac Implants*. 2014;29(Suppl):43-54.

41. Sohrabi K, Mushantat A, Esfandiari S, Feine J. How successful are small-diameter implants? A literature review. *Clin Oral Implants Res*. 2012;23(5):515-525.
42. El-Sheikh AM, Shihabuddin OF, Ghoraba SM. Two versus three narrow-diameter implants with locator attachments supporting mandibular overdentures: a two-year prospective study. *Int J Dent*. 2012;2012:285684.
43. Allum SR, Tomlinson RA, Joshi R. The impact of loads on standard diameter, small diameter and mini implants: a comparative laboratory study. *Clin Oral Implants Res*. 2008;19(6):553-559.
44. Zinsli B, Sägesser T, Mericske E, Mericske-Stern R. Clinical evaluation of small-diameter ITI implants: a prospective study. *Int J Oral Maxillofac Implants*. 2004;19(1):92-99.
45. Buser D, Mericske-stern R, Pierre Bernard JP, Behneke A, Behneke N, Hirt HP, Belser UC, Lang NP. Long-term evaluation of non-submerged ITI implants. Part 1: 8-year life table analysis of a prospective multi-center study with 2359 implants. *Clin Oral Implants Res*. 1997;8(3):161-172.
46. De Souza R, Ribeiro A, Della Vecchia M, Costa L, Cunha T, Reis A, Albuquerque Jr R. Mini vs. standard implants for mandibular overdentures: a randomized trial. *J Dent Res*. 2015;94(10):1376-1384.
47. Albrektsson T, Zarb G, Worthington P, Eriksson A. The long-term efficacy of currently used dental implants: a review and proposed criteria of success. *Int J Oral Maxillofac Implants*. 1986;1(1):11-25.
48. Mundt T, Schwahn C, Stark T, Biffar R. Clinical response of edentulous people treated with mini dental implants in nine dental practices. *Gerodontol*. 2015;32(3):179-187.
49. Javed F, Malmstrom H, Kellesarian SV, Al-Kheraif AA, Vohra F, Romanos GE. Efficacy of Vitamin D3 Supplementation on Osseointegration of Implants. *Implant Dent*. 2016;25:281–287.
50. Bouillon R, Carmeliet G, Verlinden L, van Etten E, Verstuyf A, Luderer H, Lieben L, Mathieu C, Demay M. Vitamin D: Direct effects of vitamin D metabolites on bone: Lessons from genetically modified mice. *BoneKEy Rep*. 2014;3:499.
51. Moreira ML, Neto LV, Madeira M, Lopes RF, Farias MLF. Vitamin D Deficiency and Its Influence on Bone Metabolism and Density in a Brazilian Population of Healthy Men. *J Clin Densitom*. 2018;21:91–97.
52. Mangano FG, Oskouei SG, Paz A, Mangano N, Mangano C. Low serum vitamin D and early dental implant failure: Is there a connection? A retrospective clinical study on 1740 implants placed in 885 patients. *J Dent Res Dent Clin Dent Prospect*. 2018;12:174–182.
53. Trybek G, Aniko-Wlodarczyk M, Kwiatek J, Preuss O, Brodkiewicz A, Sinicyn A, Grzywacz A. The effect of vitamin D3 on the osteointegration of dental implant. *Balt J Health Phys Act*. 2018;10:25–33.
54. Kwiatek J, Jaron A, Trybek G. Impact of the 25-Hydroxycholecalciferol Concentration and Vitamin D Deficiency Treatment on Changes in the Bone Level at the Implant Site during the Process of Osseointegration: A Prospective, Randomized, Controlled Clinical Trial. *J Clin Med*. 2021;10(3):526.
55. Papaspyridakos P, Chen CJ, Singh M, Weber HP, Gallucci G. Success criteria in implant dentistry: a systematic review. *J Dent Res*. 2012;91(3):242-248.
56. Roos J, Sennerby L, Lekholm U, Jemt T, Gröndahl K, Albrektsson T. A qualitative and quantitative method for evaluating implant success: a 5-year retrospective analysis of the Brånemark implant. *Int J Oral Maxillofac Implants*. 1997;12(4):504-514.
57. Kido H, Schulz E, Kumar A, Lozada J, Saha S. Implant diameter and bone density: effect on initial stability and pull-out resistance. *J Oral Implantol*. 1997;23(4):163-169.
58. Dierens M, Collaert B, Deschepper E, Browaeys H, Klinge B, De Bruyn H. Patient-centered outcome of immediately loaded implants in the rehabilitation of fully edentulous jaws. *Clin Oral Implants Res*. 2009;20(10):1070-1077.
59. Papaspyridakos P, Mokti M, Chen CJ, Benic GI, Gallucci GO, Chronopoulos V. Implant and prosthodontic survival rates with implant fixed complete dental prostheses in the edentulous mandible after at least 5 years: a systematic review. *Clin Implant Dent Related Res*. 2014;16(5):705-717.

Review

Motivational interviewing in promoting oral health: A literature review

Łukasz Natanek[A,B,D], Marcin Krzysztof Adamiecki[A,E], Sebastian Kłosek[F]

Department of Oral Pathology, Medical University of 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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2023;60(2):355–362

Address for correspondence
Marcin Krzysztof Adamiecki
E-mail: marcin.adamiecki@umed.lodz.pl

Funding sources
None declared

Conflict of interest
None declared

Acknowledgements
None declared

Received on April 4, 2021
Reviewed on July 7, 2021
Accepted on July 15, 2021

Published online on June 30, 2023

Abstract

Behavioral sciences are a group of disciplines that involve the study of human actions. Various behavioral models have been used in the past 50 years in behavior modification. One behavioral model currently being studied for its application in oral health is Motivational Interviewing (MI). MI is a patient-centered psychobehavioral method used in various fields of medicine and psychology to help patients change their health-affecting behaviors. Effective health promotion is important because, in developed countries, the majority of deaths and diseases are caused by chronic conditions. Controlling and treating chronic diseases requires sustained commitment. This literature review aims to describe the current methods of health promotion for oral health, based on various disease models with a thorough discussion of the psychobehavioral method known as MI, and its applications in dentistry and oral health. There is evidence that MI has applications in various health and dental areas such as oral health promotion, early childhood caries, periodontal disease, smoking cessation, and improving the quality of life during and after cancer treatment. The clinical and research limitations of this method will also be addressed. Comparing the general ideas and ethos of MI to the definition of health promotion by the World Health Organization, it can be stated that they have a common approach to promoting health.

Keywords: oral health, health promotion, motivational interviewing

Cite as
Natanek Ł, Adamiecki MK, Kłosek S. Motivational interviewing in promoting oral health: A literature review. *Dent Med Probl.* 2023;60(2):355–362. doi:10.17219/dmp/140221

DOI
10.17219/dmp/140221

Introduction

Behavioral sciences are a group of disciplines that involve the study of human actions. In dentistry, behavioral sciences focus on the clinical diagnosis and promotion of oral health using specific disease models. Thanks to behavioral sciences, our understanding of oral health has expanded from a narrow view as a 'disease-only' problem to a broader approach where oral health is understood through the biopsychosocial model — including physical, social, and psychological aspects of oral health.[1,2] Various behavioral models have been used in the past 50 years for behavior modification, although only since the mid-1990s have they been applied to dental research.[2]

One behavioral model currently being studied for its application in oral health is Motivational Interviewing (MI). MI is a patient-centered psychobehavioral method used in various fields of medicine and psychology to help patients change their health-affecting behaviors. MI has been applied in promoting oral health,[3,4,5] smoking cessation,[6,7] periodontal disease,[8] and improving the quality of life during and after cancer treatment.[9]

Effective health promotion is important because, in developed countries, the majority of deaths and diseases are caused by chronic conditions or non-communicable diseases like lung cancers and cardiovascular diseases.[10] The World Health Organization (WHO) defines health promotion as "the process of enabling people to increase control over, and to improve, their health."[11]

Controlling and treating chronic diseases requires sustained commitment and modification of established behaviors on the part of the patient.[12, 13] Oral health-related behaviors are strongly linked to other behaviors that patients use to deal with challenges in their lives. Social and cultural factors that influence these behaviors can be difficult to consider in the context of a regular dental visit. Therefore, there is a need to develop a strategy for effective oral health promotion by dentists and general health care professionals.[4]

Oral diseases such as caries, periodontal disease, and cancers are the most common chronic diseases found among people. These diseases have a significant impact on a person's quality of life and overall health. The frequent occurrence of some of these diseases (caries and periodontal disease) makes the oral cavity one of the most expensive parts of the body to treat.[14] Annual expenditures on oral diseases in the European Union (2008–2012) amounts to approximately 79 billion euro.[15]

The key problem in controlling a patient's health is not his/her lack of knowledge, but incorrect compliance with the physician's or dentist's recommendations. This key issue is complex and will be discussed in this article. Compliance with treatment recommendations is of significant value, since according to some studies, as many as 50% of patients do not take their prescribed medications, and a similar percentage of patients that take their medications, do so incorrectly.[16]

This literature review aimed to describe the current methods of health promotion for oral health, based on various disease models with a thorough discussion of the psychobehavioral method known as MI, and its applications in dentistry and oral health. The clinical and research limitations of this method will also be addressed.[3]

For this literature review, the MEDLINE database was searched through PubMed. Keywords included were: Motivational Interviewing/Motivational Interview, Oral Health, Oral Health Promotion, Caries, Early Childhood Caries (ECC), Periodontitis, and Cancer Review. Only review articles were included.

Health promotion and various disease models – biomedical and biopsychosocial

The most commonly used disease model during the 20th century is the biomedical model, where a specific pathology is the cause of the disease, and removal of the cause leads to recovery. This model assumes that every disease has a causative or pathological factor in some system or organ, and the patient is a passive recipient of treatment. Mental and emotional states are separated from other disorders of the body's function.[17] The biomedical model has its applications and greatest advances in medicine due to the success of this model, for example, through the work of Louis Pasteur and Robert Koch,[18] or the effectiveness of vaccines which prevent about 6 million deaths per year.[19] Based on this model, the patient simply needs the right information or instruction to make changes in their behavior.[17] Unfortunately, in terms of oral health, simply giving advice and providing the information is not effective enough for patients to make long-term behavior changes.[4,17,20] Often, simple advice-giving overlooks the social, economic, political, and environmental aspects of a patient's life.[4]

Another approach used to try to change a patient's behavior is to intimidate him or her with potential consequences that may arise if they fail to follow the recommendations, e.g. loss of teeth or cancer. However, fear is not an effective motivator for behavior change.[21] Scaring or stressing the patient could lead to, or contribute to chronic stress, which has significant effects on performing routine activities. People that are stressed repeat the same behaviors despite the fact that the given behavior ceased to provide a valuable effect. The awareness between behavior and results becomes impaired. This is the result of glucocorticoids, like cortisol, that negatively affect the part of the brain responsible for making the right decisions (prefrontal cortex).[22] When considering human behavior (and how to change it), it's impossible to view a problem purely from a biological or psychological perspective. The two cannot be separated and are completely

interconnected.[23] A more comprehensive disease model, currently recommended by the WHO, is the biopsychosocial model.[24,25]

Proposed by the psychiatrist George Engel in 1977, the biopsychosocial model was supposed to fill the social and psychological deficiencies of the biomedical model.[26] The model has been studied and developed since the 1970s and since 2018, about 500 articles concerning the biopsychosocial model have been published annually.[27] Despite the criticism of the biomedical model, the biopsychosocial model does not intend to replace the biomedical model but to supplement it.[27] One of the more important features of the biopsychosocial model is the recognition of the many factors that can influence behavior, of which disease (pathology), is only one. The biopsychosocial model includes psychological or mental factors, such as expectations, emotional states, beliefs, goals, etc. All these personal factors influence the patient's behavior.

In addition, this model recognizes that psychological and social factors have an impact on how the patient perceives the disease and what it means to be sick.[17] This model also has the potential to explain and predict various observations, such as functional disorders (e.g. Irritable Bowel Syndrome).[28] Non-linear and complex relationships between various factors are crucial.[28] According to this model, all activities are focused on the patient (patient/client-centered), where the patient plays a central role in his disease and treatment process. A client-centered consultation is characterized by sensitivity to the patient's social and environmental conditions. This allows the healthcare provider to include social health determinants in the consultation, which helps to motivate the patient to change their behavior more effectively. Attempts to remove the causes of oral diseases in isolation from the social situations of patients are ineffective in both the short and long term.[4] MI shares many aspects with the above-mentioned biopsychosocial model. MI is a way of helping the patient change from a psychobehavioral perspective, based on a specific dialogue and established cooperation with the patient, to discover and engage his or her reasons for making a change in their behavior.[21]

Definition and strategy of motivational interviewing

Founded by Miller in 1983 for the treatment of people with alcohol use disorder[29], MI is currently used in various fields, like dietary consultation, physical education, diabetes prevention, and oral health promotion.[30]

MI is defined as a collaboration-based conversation with special attention given to "change talk". In MI, dialogue is used as a way to strengthen personal motivation and oblige the patient to achieve a specific goal by evoking and examining his or her reasons for a change in the presence of acceptance and compassion.[21] The MI process can be understood through four themes:

– engaging – engaging with a person to cooperate, for example through open questions;
– focusing – developing and maintaining a specific direction in the dialogue about change;
– evoking – finding reasons important for the patient to make a change; and
– planning – discussing a plan that can be applied and which best suits the patient.[21]

Ambivalence is a common attitude that patients hold in relation to harmful behavior, where there are two opinions: one for and one against a particular behavior. For example, "I know that I have to brush my teeth two times per day, but I don't really eat a lot of candy". Ambivalence often accompanies us in everyday life and is normal. However, in the dental office, when the dentist argues for one side of an ambivalent statement (for example to stop smoking), the patient naturally supports the other side and therefore justifies his or her behavior ("It's only half a pack").[21]

One of the goals of MI is to help the patient to engage in "change talk" and support the parts of the ambivalent attitude that leads to a healthy change.[21] People believe what they hear themselves say[21,31], therefore, it is very helpful in resolving ambivalence to refrain from the "righting reflex", and arguing for one part of the ambivalent statement. Autonomy is an important and significant part of MI and when we engage in the "righting reflex", the patient's autonomy is not honored. Medical personnel should refrain from straightening or repairing for the patient what is not correct in his behavior. Respecting a patient's autonomy is as simple as asking whether one can raise a particular topic to discuss, whereas speaking in a patronizing way or tone is not. This also includes advising without permission or forcibly saying what a patient must or must not do.[21]

Often, when people feel that their freedom to make a choice is threatened, their reaction is to justify their freedom which brings them to an internal balance, where this freedom of decision-making is not affected. This is known as reactance. Even if the decision is harmful to the person, freedom of choice is more important.[32] Another way of looking at autonomy is accepting that people can decide and make choices about the course of their lives. Doctors can inform, advise, and even warn, but ultimately the patient decides what to do. Recognizing this is a key element in facilitating behavior change. Human nature resists being forced. Accepting the rights and freedoms of others without undue interference enables change.[31]

When attempting to elicit dialogue with a patient, open questions are one of the main elements in MI. Open questions allow the patient to think about the subject before answering, leaving him/her the choice of the direction in which the conversation could go. And it will probably be a direction that is important for the patient.[31] Examples

of such open questions are those that touch upon desire, ability, reason, and need for change:
– desire – Why do you want to make this change?
– ability – How can you do it to succeed?
– reason – What are the three best reasons to do this?
– need – How important is a change for you and why?[31]

Summing up the abovementioned principles, a patient changes their behavior because it is their reasons that lead to change and not someone else's. Applying a MI-based approach leads to an understanding of the patient's perspective, supporting the correct side of ambivalence, and abstaining from solving a patient's problems for them. By using open questions, one can create a specific vision for change (focusing), and when the patient will be ready for it, planning a way how this change can be achieved.[21]

Motivational interviewing and oral health

There is evidence that MI has applications in various health and dental areas such as oral health promotion,[4,33] ECC,[5,34] periodontal disease,[8] smoking cessation,[6,7] and improving the quality of life during and after cancer treatment.[9]

Researchers from Australia, evaluated various health promotion models and their effectiveness, including clinical prevention and health education, psychological counseling, and MI.[4] Where clinical prevention and health sciences are simple information and advice given to a passive patient. Based on the results from the authors' review, "advice giving" has poor efficiency. A review of the research on psychological counseling indicated that this approach has little impact on oral health and eating habits, and only increases the use of xylitol and fluoride pills. The authors analyzed nine studies related to MI, which showed that changes in behavior caused by MI do not disappear with time.[4] MI not only has positive results with specific problems but also seems to positively affect the patient in a wider and socially significant manner. In addition, MI takes less time than the other methods tested, which makes it more profitable. The authors comment that the reviewed research confirms the complex nature of 'behavioral change', which supports the need for effective ways to promote health. The authors suggest that studying oral disease in isolation from the patient's life and social circumstances is not effective. There is a need to create and apply oral health promotion that focuses more on the causes of diseases and on respecting the patient's expertise regarding their life. Recognizing the broader context that triggers certain behaviors can help healthcare professionals be more effective in working with patients to help them change their harmful behaviors and habits.[4]

A 2017 systematic review examined the use of MI in dentistry.[33] The review analyzed eight studies that met the search criteria. One study examined the effect of MI on parents of children at increased risk of caries. The intervention consisted of one session and six telephone conversations. Children in the MI group had more than half the number of caries than the control group. The quality of the remaining studies varied and did not show long-term changes in oral behavior. The authors concluded that understanding and accepting patients in the context of oral health, without judging, helps create a therapeutic environment that supports oral health promotion. Teaching medical staff about health psychology would allow for more effective promotion of oral health. Although this review was based mainly on MI, out of the eight studies it included, only one was related directly to MI.[33]

Two recent systematic reviews and meta-analyses looked at the applications of MI in the treatment and prevention of ECC. Applying behavioral techniques in the management of ECC is a challenge because they are used indirectly: the behavior change must first go through the parent and secondly be passed on to the child. A review and meta-analysis found eight studies from 2004 to 2018, where six had positive results.[34] In the meta-analysis of three studies, the results were found to be inconclusive and had high/significant heterogeneity. A majority of the studies used only one MI session, and an appropriate dose of MI could not be established. The authors noted that a limitation of their study was that few studies reviewed had proper quality or a standardized methodology, along with high heterogeneity.[34]

Another review and meta-analysis of MI in the prevention of ECC, conducted by Colvara et. al. in 2020, performed a broader search (for example, no language restrictions) using different search criteria.[5] They included 14 studies in the systematic review and eight in the meta-analysis. Four studies in the systematic review found MI had a protective effect against caries. In the meta-analysis, a subgroup effect was found where patients with high caries experience, benefited more from the intervention as MI prevented more caries than in patients who had a lower caries experience. Compared with the previous meta-analysis, the studies assessed in this meta-analysis had low heterogeneity. The authors conclude that there was significant methodological variety in the studies reviewed, for example with the design and implementation of the intervention, which posed problems. The authors also noted that only three studies (out of 14) had an oral professional as the counselor administering MI. There were also some differences in what constituted a control group, with various definitions of "conventional oral health education". The authors conclude that MI could be recommended as part of a preventive strategy, especially in groups of patients with a high disease burden.[5]

A systematic review conducted by Kopp et al. in 2017 looked at the effectiveness of MI as an adjunctive therapy in the treatment of periodontal disease (oral hygiene instruction with scaling and root planing [SRP]) and wheth-

er the duration of MI treatment had any impact.[8] The review found five studies, where three showed positive results (one with MI alone, two where MI was mixed with the theory of self-efficacy). The authors made a number of important observations. In the study where the therapy was conducted by a counselor specializing in MI, the intervention did not produce positive results, whereas studies with hygienists and dental students trained in MI showed more positive results. According to the authors, this may be because patients have more confidence in medical staff. The authors suggest that more long-term studies should be conducted and that subsequent studies should have a standardized method of applying MI, e.g. with a textbook, and should not be combined with other types of therapy. Despite these observations, the authors conclude that MI may have a positive effect on the treatment of periodontal disease but more long-term studies should be conducted.[8]

Since 2008, the Cochrane Library has been publishing reviews of MI on smoking cessation.[35] The most recent review from 2019 looked at 37 studies with a total of over 15,000 participants. The participants in the studies varied significantly (young people, homeless people, incarcerated individuals, etc.). The studies compared also greatly varied with MI being conducted in one to twelve sessions and lasting from five minutes to more than 5 hours. The authors conclude that there is insufficient evidence that MI helps people with smoking cessation, but that the treatment effect may be low due to bias, imprecision, and inconsistency within the trials.[6]

In 2015, the Cochrane Database of Systematic Reviews also reviewed the effectiveness of MI in smoking cessation. The authors examined 28 studies, with a total of over 16,000 participants. In this systematic review, MI was compared with short advice or normal care.[7] The intervention was performed by family doctors, nurses, or psychologists. Conversations lasting less than twenty minutes had a better effect than those lasting longer, and the number of interventions had no statistical significance. The review confirms the results of other studies that therapy provided by the attending physician gives better results than a nurse or counselor. Interventions carried out by phone or in person in the office had comparable results. The review confirmed that MI has a statistically significant advantage over the usual advice to quit smoking. But care should be taken in interpreting the results because the quality of evidence is of medium quality. Training of the "counselors" in MI ranged from two to forty hours. The authors conclude that there is a need for greater consistency and clarity of methods in the conducted trials.[7]

Spencer et al. analyzed the use of MI with cancer patients. The review included fifteen studies that concerned two groups of patients, one during treatment and the other after cancer treatment.[9] The authors also divided the studies into three main categories: lifestyle improvement, psychosocial support, and self-management of cancer-related symptoms. The types of cancer studied mainly concerned breast cancer, but also head and neck cancer, colorectal cancer, or any type of cancer. In the studies, the intervention was carried out by MI-trained nurses, dietitians, MI counselors, and former cancer survivors. Lifestyle improvements included improving diet and increasing physical activity. According to the authors, the best results were found in the lifestyle improvement category, but in the other two categories, the results were promising. In the lifestyle improvement category, the study confirmed positive results with healthier eating (more vegetables and fruits) and increased physical activity in groups where MI was used. The authors note that many participants left the study, but this was possibly due to the high intensity of physical exercise required.[9]

Regarding smoking cessation, one study of patients undergoing cancer treatment led to a short-term cessation of smoking after using MI. A second study showed an increased number of attempts to quit smoking. A third head and neck cancer study showed positive results where the majority (68%) of participants quitting smoking, and this result persisted for a year. Although, this study did not have a control group. In the category of psychosocial support, the authors found that MI reduces the effect of emotional stress associated with cancer, according to surveys completed by participants. Respondents reported a positive impact of MI and increased optimism and acceptance of the diagnosis and disease.[9]

In the category of self-management of cancer-related symptoms, MI has been proven effective in reducing the impact of pain on daily functioning. According to the authors, MI is easily adapted to situations where resources and time are limited.[9] There are individual studies with good results, but there were many concerns, for example, the way MI was used (in person, by phone, and attendance), regarding quality controls and the evaluation of results. In most studies, the intervention was done over the phone, not in person. The authors add that MI focuses on letting the patient develop solutions for changing behavior in everyday life. And so, empowering patients and generating motivation is important for those who have been overwhelmed by cancer or its treatment.[9]

Summaries and details of the abovementioned articles can be found in Table 1.

Need for the standardization of motivational interviewing

Frost et al. analyzed over 100 articles on the effectiveness of MI in the fields of health and social care.[3] MI was most effective in stopping unhealthy behaviors such as binge drinking, reducing the amount and frequency of alcohol consumption, smoking cessation, and substance abuse. Based on the authors' review of the literature, the

Table 1. Impact of motivational interviewing on oral health

Reference	Type of study	Number of studies	Problem reviewed	Interventions analyzed	Conclusions
Yevlahova et al. (2009)	Systematic Review	32	Oral health promotion	Clinical prevention and health education Motivational interviewing Counselling Behaviour change models	Need to shift away from the Biomedical Model and focus on social determinants of oral health. MI is most effective in altering clients' behaviours.
Kay et al. (2017)	Systematic Review	8	Oral Health promotion	Motivational Interviewing or Based on Motivational Interviewing	MI may be useful in the dental setting and should be researched further.
Colvara et al. (2021)	Systematic Review and Meta-Analysis	Systematic Review: 14 Meta-analysis: 8	Early Childhood Caries (ECC)	Motivational Interviewing or Based on Motivational Interviewing	Interventions based on MI are effective in the prevention of ECC (especially in populations with high caries experience). Studies had a wide methodological variety.
Faghihian et al. (2020)	Systematic Review and Meta-Analysis	Systematic Review: 8 Meta-analysis: 3	Early Childhood Caries (ECC)	Motivational Interviewing	MI is as effective as Dental Health Education (meta-analysis), more evidence is needed to assess the impact of MI on ECC.
Kopp et al. (2017)	Systematic Review	5	Periodontal Disease	Motivational Interviewing as an adjunct to periodontal therapy	MI as an adjunct to periodontal therapy might have a positive influence but further long-term studies are needed.
Lindson et al. (2019)	Systematic Review	37	Smoking Cessation	Motivational Interviewing	Not enough information available to conclude if MI helps with smoking cessation. More research is needed.
Lindson-Hawley et al. (2015)	Systematic Review	28	Smoking Cessation	Motivational Interviewing	MI has a statistically significant advantage over simple advice giving for smoking cessation, but the evidence is of medium quality.
Spencer et al. (2016)	Systematic Review	15	Lifestyle behaviours Psychosocial outcomes Cancer-related symptom management	Motivational Interviewing	MI is a promising intervention for promoting behaviour change in a variety of cancer types and treatment stages. Difficult to assess the application of MI in cancer populations due to poor study design.

evidence is inconclusive or of poor quality in the field of health promotion. For example, there are low-quality results that MI is effective for weight loss but there is moderate evidence that MI is effective in increasing physical activity in people with chronic diseases. The authors pointed out that MI is covered in the National Institute for Health Care and Excellence (NICE) guidelines as a potentially effective intervention for behavior modification, though not in every situation. The reason may be the lack of requirements in official MI training. In addition, MI is applied without monitoring for competency and this lack of competence can affect results. Often, studies do not describe what training in MI had been given. Therefore, there is the Motivational Interviewing Treatment Integrity (MITI) code whose purpose is to establish standard guidelines for the application of MI in practice.[3]

There is an international organization, Motivational Interviewing Network of Trainers (MINT), which operates in 26 languages, and in 35 countries, including Poland. The organization aims to promote good MI practice and MI research and training. It supports the development of knowledge and skills of its members through meetings as well as the open sharing of sources and publications. Thanks to this organization, there is support for experienced and non-experienced users of MI, as well as researchers.[36]

More information on MI is available from various sources. Some textbooks for dental students recommend using MI.[37] On the British Medical Journal (BMJ) website there is a scientific module that covers MI.[38] There is the Polish Society for Motivating Dialogue (PTTM) in Poland, where one can find more information.[39] The Center for Addiction and Mental Health (CAMH) in Toronto, Canada has films showing sample conversations between a dentist and a patient using the MI approach.[40]

Conclusions

Many psychological approaches are currently being investigated as potential methods to help patients make effective changes in their behaviors to improve their health. This is important because the psychological and biological aspects are inseparable from one another. MI is one such psychological approach. Currently used and researched in many areas of medicine, it has the potential

application in the promotion of oral health. However, research results have not always been positive. Despite this, MI is recommended and warrants more research. The MI studies likely have substantial flaws, including studies that do not show MI efficacy. More research with higher standards is needed. Comparing the general ideas and ethos of MI to the definition of health promotion by the WHO, it can be stated that they have a common approach to promoting health. MI has many positive clinical aspects that make it attractive in the dental setting when a patient's behavior should be modified.

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

Łukasz Natanek https://orcid.org/0000-0002-9133-6188
Marcin Krzysztof Adamiecki https://orcid.org/0000-0001-6644-7057
Sebastian Kłosek https://orcid.org/0000-0001-9694-0950

References

1. Lee JY, Watt RG, Williams DM, Giannobile WV. A New Definition for Oral Health. *J Dent Res*. 2016;96(2)125-127. doi:10.1177/0022034516682718
2. McGrath C. Behavioral Sciences in the Promotion of Oral Health. *J Dent Res*. 2019;98(13):1418–1424. doi:10.1177/0022034519873842
3. Frost H, Campbell P, Maxwell M, et al. Effectiveness of Motivational Interviewing on Adult Behaviour Change in Health and Social Care Settings: A Systematic Review of Reviews. *PLoS ONE*. 2018;13(10):e0204890. doi:10.1371/journal.pone.0204890
4. Yevlahova D, Satur J. Models for Individual Oral Health Promotion and Their Effectiveness: A Systematic Review. *Aust Dent J*. 2009;54(3):190–197. doi:10.1111/j.1834-7819.2009.01118.x
5. Colvara BC, Faustino-Silva DD, Meyer E, Hugo FN, Celeste RK, Hilgert JB. Motivational Interviewing for Preventing Early Childhood Caries: A Systematic Review and Meta-Analysis. *Community Dent Oral Epidemiol*. 2021;49(1):10–16. doi:10.1111/cdoe.12578
6. Lindson N, Thompson TP, Ferrey A, Lambert JD, Aveyard P. Motivational Interviewing for Smoking Cessation (Review). *Cochrane Database Syst Rev*. 2019(7):CD006936. doi:10.1002/14651858.CD006936.pub4
7. Lindson-Hawley N, Thompson TP, Begh R. Motivational Interviewing for Smoking Cessation (Review). *Cochrane Database Syst Rev*. 2015(3):CD006936. doi:10.1002/14651858.CD006936.pub3
8. Kopp SL, Ramseier CA, Ratka-Krüger P, Woelber JP. Motivational Interviewing as an Adjunct to Periodontal Therapy-A Systematic Review. *Front Psychol*. 2017;8(279):1–9. doi:10.3389/fpsyg.2017.00279
9. Spencer JC, Wheeler SB. A Systematic Review of Motivational Interviewing Interventions in Cancer Patients and Survivors. *Patient Educ Couns*. 2016;99(7):1099–1105. doi:10.1016/j.pec.2016.02.003
10. World Health Organization. The Top 10 Causes of Death. http://www.who.int/mediacentre/factsheets/fs310/en. Accessed March 21, 2021.
11. World Health Organization. Ottawa Charter for Health Promotion, 1986. http://www.euro.who.int/__data/assets/pdf_file/0004/129532/Ottawa_Charter.pdf. Accessed March 21, 2021.
12. Lundahl B, Moleni T, Burke BL, Butters R, Tollefson D, Butler C, Rollnick S. Motivational Interviewing in Medical Care Settings: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Patient Educ Couns*. 2013;93(2):157–168. doi:10.1016/j.pec.2013.07.012
13. Rollnick S, Butler CC, McCambridge J, Kinnersley P, Elwyn G, Resnicow K. Consultations about changing behaviour. *BMJ-Brit Med J*. 2005;331:961-963. doi:10.1136/bmj.331.7522.961
14. Jin LJ, Lamster IB, Greenspan JS, Pitts NB, Scully C, Warnakulasuriya, S. Global Burden of Oral Diseases: Emerging Concepts, Management and Interplay with Systemic Health. *Oral Dis*. 2016;22(7):609–619. doi:10.1111/odi.12428
15. Better Oral Health European Platform. The State of Oral Health in Europe. http://www.oralhealthplatform.eu/wp-content/uploads/2015/09/Report-the-State-of-Oral-Health-in-Europe.pdf. Accessed March 21, 2021.
16. Usherwood T. Encouraging Adherence to Long-Term Medication. *Aust Prescr*. 2017;40(4):147–150. doi:10.18773/austprescr.2017.050
17. Wade DT, Halligan PW. Do Biomedical Models of Illness Make for Good Healthcare Systems? *BMJ-Brit Med J*. 2004;329(7479):1398–1401. doi:10.1136/bmj.329.7479.1398
18. Immunization Action Coalition. Historic Dates and Events Related to Vaccines and Immunization. https://www.immunize.org/timeline. Accessed March 21, 2021.
19. Bulletin of the World Health Organization. Vaccination Greatly Reduces Disease, Disability, Death and Inequity Worldwide. https://www.who.int/bulletin/volumes/86/2/07-040089.pdf. Accessed March 21, 2021.
20. Kay E, Vascott D, Hocking A, Nield H, Dorr C, Barrett H. A Review of Approaches for Dental Practice Teams for Promoting Oral Health. *Community Dent Oral Epidemiol*. 2016;44(4)313–330. doi:10.1111/cdoe.12220
21. Miller WR, Rollnick S. *Motivational Interviewing: Helping People Change*. 3rd ed. New York, USA: Guilford Press; 2013.
22. Schwabe L, Wolf OT. Stress Prompts Habit Behavior in Humans. *J Neurosci*. 2009;29(22):7191–7198. doi:10.1523/JNEUROSCI.0979-09.2009
23. Sapolsky RM. *Behave: The Biology of Humans at Our Best and Worst*. New York, USA: Penguin Press; 2017.
24. World Health Organization. Towards a Common Language for Functioning, Disability and Health ICF. http://www.who.int/classifications/icf/training/icfbeginnersguide.pdf. Accessed March 21, 2021.
25. Kostanjsek N. Use of the International Classification of Functioning, Disability and Health (ICF) as a Conceptual Framework and Common Language for Disability Statistics and Health Information Systems. *BMC Public Health*. 2011;11(Suppl 4):2–7. doi:10.1186/1471-2458-11-S4-S3
26. Engel GL. The Need for a New Medical Model: A Challenge for Biomedicine. *Science*. 1977;196(4286): 129–196. doi:10.1126/science.847460
27. Wade DT, Halligan PW. The Biopsychosocial Model of Illness: A Model Whose Time Has Come. *Clin Rehabil*. 2017;31(8):995–1004. doi:10.1177/0269215517709890
28. Wade D. 2015. Rehabilitation - A New Approach. Part Two: The Underlying Theories. *Clin Rehabil*. 2015;29(12):1145–1154. doi:10.1177/0269215515601175
29. Miller WR. Motivational Interviewing with Problem Drinkers. *Behav Psychother*. 1983;11:147-172.
30. Martins RK, McNeil DW. Review of Motivational Interviewing in Promoting Health Behaviors. *Clin Psychol Rev*. 2009;29(4):283–293. doi:10.1016/j.cpr.2009.02.001
31. Rollnick S, Miller WR, Butler CC. *Motivational Interviewing in Health Care: Helping Patients Change Behavior*. New York, USA: Guilford Press; 2008.
32. Dillard JP, Shen L. On the Nature of Reactance and Its Role in Persuasive Health Communication. *Commun Monogr*. 2005;72(2):144–168. doi:10.1080/03637750500111815
33. Kay EJ, Vascott D, Hocking A, Nield H. Motivational Interviewing in General Dental Practice: A Review of the Evidence. *Br Dent J*. 2017;221(12):785–791. doi:10.1038/sj.bdj.2016.952

34. Faghihian R, Faghihian E, Kazemi A, Tarrahi MJ, Zakizade M. Impact of Motivational Interviewing on Early Childhood Caries: A Systematic Review and Meta-Analysis. *J Am Dent Assoc.* 2020;151(9):650–659. doi:10.1016/j.adaj.2020.06.003
35. Cochrane Library. Motivational Interviewing for Smoking Cessation. https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD006936.pub4/information#whatsNew. Accessed March 21, 2021.
36. Motivational Interviewing Network of Trainers. Motivational Interviewing Resources. https://motivationalinterviewing.org/motivational-interviewing-resources. Accessed March 21, 2021.
37. Kidd E, Fejerskov O. *Essentials of Dental Caries*. 4th ed. Oxford, UK: Oxford University Press; 2016.
38. BMJ Learning. Motivational Interviewing in Brief Consultations. https://new-learning.bmj.com/course/10051582. Accessed March 21, 2021.
39. Polskie Towarzystwo Terapii Motywującej. http://pttm.org.pl. Accessed March 21, 2021.
40. Teach Project, Motivational Interviewing. Centre for Addiction and Mental Health. https://www.youtube.com/user/teachproject. Accessed March 21, 2021.

Dental

and Medical Problems