# Dental and Medical Problems

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

2024, Vol. 61, No. 4 (July–August)

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

Wroclaw Medical University

# Dental and Medical Problems

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

**BIMONTHLY**
**2024, Vol. 61, No. 4**
**(July–August)**

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



**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

**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: PRINT PROFIT Sp. z o.o.

Dental
and Medical Problems

BIMONTHLY 2024, Vol. 61, No. 4 (July–August)

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

# Contents

## Reviews



## Research letter

Comment

# Sleep disorders and cardiovascular risk: Focusing on sleep fragmentation

Helena Martynowicz[1,A–D], Adam Wichniak[2,E,F], Mieszko Więckiewicz[3,A,E,F]

[1] Department and Clinic of Internal Medicine, Occupational Diseases, Hypertension, and Clinical Oncology, Wroclaw Medical University, Poland
[2] Third Department of Psychiatry, Institute of Psychiatry and Neurology, Warsaw, Poland
[3] Department of Experimental Dentistry, Wroclaw Medical University, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(4):475–477

**Address for correspondence**
Helena Martynowicz
E-mail: helena.martynowicz@umw.edu.pl

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on February 12, 2024
Reviewed on February 16, 2024
Accepted on February 26, 2024

Published online on March 22, 2024

**Cite as**
Martynowicz H, Wichniak A, Więckiewicz M. Sleep disorders and cardiovascular risk: Focusing on sleep fragmentation. *Dent Med Probl.* 2024;61(4):475–477. doi:10.17219/dmp/185395

**DOI**
10.17219/dmp/185395



**Keywords:** sleep, hypertension, cardiovascular risk, vascular endothelium, arousals

**In this comment, we explored the link between sleep fragmentation and the cardiovascular risk, considering various sleep disorders and methodologies for assessing sleep fragmentation.**

It is generally known that sleep deprivation increases the cardiovascular (CV) and metabolic risk. Additionally, some data indicates that prolonged sleep is also associated with such risk.[1] In addition to sleep duration, sleep quality is also essential in the assessment of the CV risk. The continuity of sleep is one of its crucial features that contributes to effective rest and refreshment. Sleep may be interrupted by arousals and awakenings, which can result in prolonged wakefulness after sleep onset (WASO). Therefore, it is important to determine the threshold for the number of arousals and awakenings during sleep to consider them a normal phenomenon. According to the American Academy of Sleep Medicine (AASM) definition, an arousal is an abrupt shift in electroencephalography (EEG) frequencies, including alpha, theta and/or frequencies greater than 16 Hz (but not spindles), that lasts at least 3 s, with at least 10 s of stable sleep preceding the change.[2] The scoring of arousals during rapid eye movement (REM) sleep requires a concurrent increase in the submental electromyography (EMG) lasting at least 1 s.[2] Arousals, which can be accompanied by respiratory events, periodic limb movements in sleep (PLMS) or sleep bruxism events, are usually followed by autonomic activation, resulting in increased heart rate and blood pressure. Nevertheless, they can also occur spontaneously or be elicited by pain, light, noise, or a change in temperature. The arousal index (ArI), counted as the number of arousals per sleep hour, is frequently considered to quantify sleep fragmentation.[3] Sleep fragmentation, characterized by repetitive interruptions of sleep, is one of the factors contributing to excessive daytime sleepiness.

The apnea–hypopnea index (AHI) is generally considered an imperfect indicator of obstructive sleep apnea (OSA) severity. It is strongly believed that oxygen saturation parameters (i.e., the mean oxygen saturation, the oxygen desaturation index (ODI), the nadir oxygen saturation, and the percentage of sleep with oxygen saturation <90%) are more effective predictors of CV complications than the index of respiratory events.[4] It is worth noting that

if the duration of apneas is long, the AHI values decrease, leading to an underestimation of OSA severity. Recent studies have also emphasized the importance of sleep fragmentation. Shahrbabaki et al. showed sleep fragmentation to be a long-term risk factor for all-cause and CV mortality, indicating that the association between mortality and sleep fragmentation was more pronounced in women than in men.[5] Interestingly, women usually report a significantly worse sleep quality as compared to men.[6] Repetitive arousals disrupt the circadian rhythm of the CV system through the modification of blood pressure patterns. The physiological "deeper" pattern measured in ambulatory blood pressure monitoring (ABPM) shifts to the "non-deeper" or "reverse deeper" pattern due to the lack of physiological decreases in blood pressure or due to increases in blood pressure during sleep, respectively.[1] Among the main consequences of sleep fragmentation there are insulin resistance, lipid profile dysregulation and the overdrive of the sympathetic system, leading to a sustained increase in daytime blood pressure. Sleep fragmentation is a pivotal stimulus to sympathetic activation, resulting in ArI being correlated with the sympathetic overdrive much closer than AHI.[7] This crucial observation may explain the increased CV and metabolic risk in patients with sleep fragmentation. Another mechanism may involve endothelium dysfunction leading to an increase in blood pressure. Indeed, recent studies have shown a link between sleep fragmentation and hypertension.[8] Consequently, a high ArI and a low percentage of time spent in stage 3 non-rapid eye movement (NREM) sleep (N3) are associated with a greater coronary artery calcification burden.[9] Zhang et al. showed that a high ArI is a risk factor for increases in the left atrial diameter and correlates with cardiac remodeling.[10] Recently, it has been found that sleep fragmentation can increase QT interval variability during arousal, which is associated with an increased all-cause and CV mortality.[11]

The current issue to be dealt with is defining the cut-off value that leads to an increase in the CV risk. The task has been extremely difficult due to the lack of sufficient data until now. The cut-off point for a normal arousal is fluid and depends on age.[12,13] An ArI of >32 events/h was reported to increase the risk of coronary plaque development as compared to controls (ArI < 32 events/h).[14] However, more studies on sleep fragmentation are needed to effectively determine the cut-off point for ArI in relation to the CV risk.

Another question is whether the significance of different arousals is equal. Most studies do not consider different polysomnographic types of arousals (Fig. 1), as such polysomnography assessment is difficult and time-consuming. Respiratory arousals following respiratory events in sleep-disordered breathing patients are most commonly studied. Unfortunately, the data concerning arousals evoked by PLMS or sleep bruxism is limited. In a study by Kanclerska et al., it was observed that the PLMS index was increased in hypertensive patients as compared to normotensive controls, but the ArI related to PLMS was

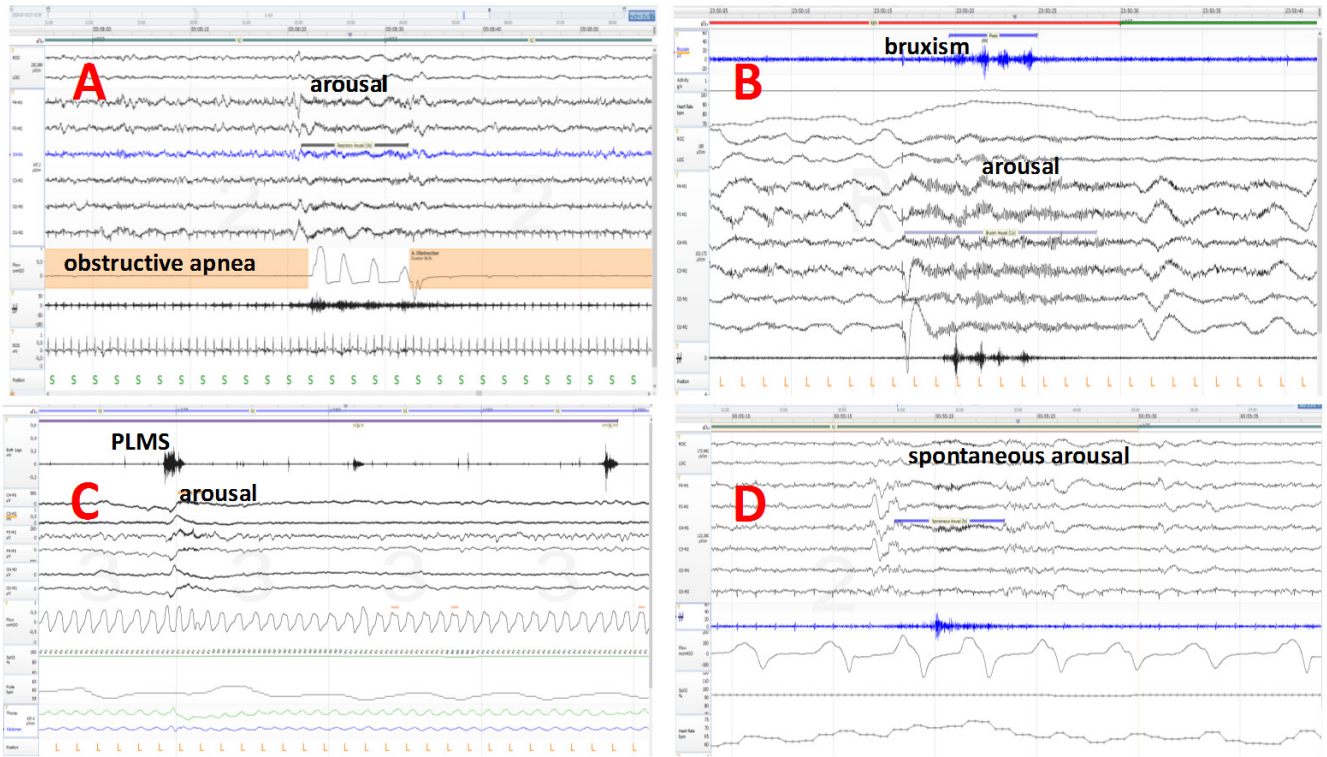


**Fig. 1.** Arousals in polysomnography

A – respiratory arousal; B – sleep bruxism arousal; C – periodic limb movements in sleep (PLMS) arousal; D – spontaneous arousal.

similar in normotensive and hypertensive patients, suggesting no significant sleep fragmentation due to PLMS in hypertensives; on the other hand, the ArI related to sleep bruxism was decreased in hypertensive patients as compared to normotensive controls.[15] Sleep bruxism is considered a physiological phenomenon; however, in some cases, it can be associated with systemic inflammation and excessive daytime sleepiness.[16] An increased frequency of arousals was demonstrated in repetitive sleep bruxism.[17] Although the treatment of bruxism is still not fully effective,[18] pharmacological therapy with sleep-promoting drugs, such as opipramol, may decrease ArI in patients with sleep bruxism.[19]

The last but not least issue is the quantification of sleep fragmentation. Sleep fragmentation can be determined with the sleep fragmentation index (SFI), calculated as the total number of awakenings and shifts to stage 1 NREM sleep (N1) divided by the total sleep time (TST). The most commonly used parameter is ArI, defined as the number of arousals per hour of sleep. However, both SFI and ArI do not consider arousal duration, unlike a new parameter called the arousal burden (AB), indicating the cumulative duration of all arousals relative to TST.[5] Unfortunately, none of these parameters includes all awakenings ("long arousals" lasting >15 s, which are also related to excessive daytime sleepiness).[20] Finally, the definitions of arousals include only EEG disruption (and additionally an increase in the EMG tone during REM sleep), but not autonomic activation, such as increases in the heart rate or blood pressure.

The current paper has addressed the important link between the CV risk and sleep fragmentation. In this context, the number of gaps and missing data was also revealed. Sleep fragmentation may represent a promising marker in identifying subjects at risk; however, new data on sleep fragmentation parameters, cut-off points, as well as larger data sets from clinical trials, are needed.

## ORCID iDs

Helena Martynowicz https://orcid.org/0000-0003-1283-8460
Adam Wichniak https://orcid.org/0000-0002-5352-601X
Mieszko Wieckiewicz https://orcid.org/0000-0003-4953-7143

## References

1. Wang C, Bangdiwala SI, Rangarajan S, et al. Association of estimated sleep duration and naps with mortality and cardiovascular events: A study of 116 632 people from 21 countries. *Eur Heart J*. 2019;40(20):1620–1629. doi:10.1093/eurheartj/ehy695
2. Berry RB, Brooks R, Gamaldo CE et al. for the American Academy of Sleep Medicine. *The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Version 2.1*. Darien, IL: American Academy of Sleep Medicine; 2014. https://aasm.org. Accessed November 6, 2023.
3. Smurra MV, Dury M, Aubert G, Rodenstein DO, Liistro G. Sleep fragmentation: Comparison of two definitions of short arousals during sleep in OSAS patients. *Eur Respir J*. 2001;17(4):723–727. doi:10.1183/09031936.01.17407230
4. Labarca G, Vena D, Hu WH, et al. Sleep apnea physiological burdens and cardiovascular morbidity and mortality. *Am J Respir Crit Care Med*. 2023;208(7):802–813. doi:10.1164/rccm.202209-1808OC
5. Shahrbabaki SS, Linz D, Hartmann S, Redline S, Baumert M. Sleep arousal burden is associated with long-term all-cause and cardiovascular mortality in 8001 community-dwelling older men and women. *Eur Heart J*. 2021;42(21):2088–2099. doi:10.1093/eurheartj/ehab151
6. Seweryn P, Orzeszek SM, Waliszewska-Prosół M, et al. Relationship between pain severity, satisfaction with life and the quality of sleep in Polish adults with temporomandibular disorders. *Dent Med Probl*. 2023;60(4):609–617. doi:10.17219/dmp/171894
7. Taylor KS, Murai H, Millar PJ, et al. Arousal from sleep and sympathetic excitation during wakefulness. *Hypertension*. 2016;68(6):1467–1474. doi:10.1161/HYPERTENSIONAHA.116.08212
8. Ren R, Zhang Y, Yang L, Shi Y, Covassin N, Tang X. Sleep fragmentation during rapid eye movement sleep and hypertension in obstructive sleep apnea. *J Hypertens*. 2023;41(2):310–315. doi:10.1097/HJH.0000000000003332
9. Lutsey PL, McClelland RL, Duprez D, et al. Objectively measured sleep characteristics and prevalence of coronary artery calcification: the Multi-Ethnic Study of Atherosclerosis Sleep study. *Thorax*. 2015;70(9):880–887. doi:10.1136/thoraxjnl-2015-206871
10. Zhang B, Lu S, Guo H, Xu J, Xiao Z, Tang J. Relationship between ODI and sleep structure of obstructive sleep apnea and cardiac remodeling. *Sleep Breath*. 2023. doi:10.1007/s11325-023-02872-7
11. Shahrbabaki SS, Linz D, Redline S, Stone K, Ensrud K, Baumert M. Sleep arousal-related ventricular repolarization lability is associated with cardiovascular mortality in older community-dwelling men. *Chest*. 2023;163(2):419–432. doi:10.1016/j.chest.2022.09.043
12. Bonnet MH, Arand DL. EEG arousal norms by age. *J Clin Sleep Med*. 2007;3(3):271–274. PMID:17561594. PMCID:PMC2564772.
13. Jaimchariyatam N, Rodriguez CL, Budur K. Sleep-related cortical arousals in adult subjects with negative polysomnography. *Sleep Breath*. 2015;19(3):989–996. doi:10.1007/s11325-014-1090-x
14. Lu M, Yu W, Wang Z, Huang Z. Association between arousals during sleep and subclinical coronary atherosclerosis in patients with obstructive sleep apnea. *Brain Sci*. 2022;12(10):1362. doi:10.3390/brainsci12101362
15. Kanclerska J, Wieckiewicz M, Nowacki D, et al. Sleep architecture and vitamin D in hypertensives with obstructive sleep apnea: A polysomnographic study. *Dent Med Probl*. 2024;61(1):43–52. doi:10.17219/dmp/172243
16. Kostrzewa-Janicka J, Jurkowski P, Zycinska K, Przybyłowska D, Mierzwińska-Nastalska E. Sleep-related breathing disorders and bruxism. *Adv Exp Med Biol*. 2015;873:9–14. doi:10.1007/5584_2015_151
17. Michalek-Zrabkowska M, Wieckiewicz M, Smardz J, et al. Determination of inflammatory markers, hormonal disturbances, and sleepiness associated with sleep bruxism among adults. *Nat Sci Sleep*. 2020;12:969–979. doi:10.2147/NSS.S268470
18. 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
19. 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
20. Schwartz DJ, Moxley P. On the potential clinical relevance of the length of arousals from sleep in patients with obstructive sleep apnea. *J Clin Sleep Med*. 2006;2(2):175–180. PMID:17557492.

# Neglected area of oral cancer: A word about the International Agency for Research on Cancer (IARC) "Handbook of Oral Cancer Prevention"

Irena Duś-Ilnicka[A,B,D,F]

Department of Oral Pathology, Faculty of Dentistry, Wroclaw Medical University, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(4):479–480

**Address for correspondence**
Irena Duś-Ilnicka
E-mail: irena.dus-ilnicka@umw.edu.pl

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on June 18, 2024
Reviewed on June 20, 2024
Accepted on June 25, 2024

Published online on August 16, 2024

**Keywords:** oral cancer, leukoplakia, oral potentially malignant disorders

**The International Agency for Research on Cancer (IARC) "Handbook of Oral Cancer Prevention", vol. 19, provides a thorough and comprehensive evidence-based evaluation of primary and secondary prevention interventions for oral cancer.**

In order to provide a due introduction to "Handbook of Oral Cancer Prevention", vol. 19, released by the International Agency for Research on Cancer (IARC) in November 2023, it is relevant to start with a short history of the IARC handbooks programme.[1] This series was launched in 1995 by Dr. Paul Kleihues, the former director of IARC. The IARC handbooks evaluate the evidence on the cancer-preventive potential of various agents and interventions for primary and secondary prevention. Previous volumes have covered the issues of Breast Cancer Screening (Handbook 15), Absence of Excess Body Fatness (Handbook 16), Colorectal Cancer Screening (Handbook 17), and Cervical Cancer Screening (Handbook 18).

Here we report on the IARC first evaluation regarding Oral Cancer Prevention, presented in Handbook 19 (Fig. 1). A working group comprising 25 independent international experts from 12 countries (South Africa, United States of America, Taiwan (China), United Kingdom, India, Switzerland, Japan, Brazil, Panama, France, Colombia, and Malaysia) met remotely between September and December 2021. For the first time, this handbook synthesizes all the available evidence related to the primary and secondary prevention of oral cancer.

The volume begins with two preambles, one for primary and one for secondary prevention. These preambles establish the methodological procedures for developing the handbook, i.e., criteria for including studies, definitions, the selection process for interventions, and outcomes. The methods used to select cancer prevention studies are described, along with the criteria used to assess the strength of evidence in each evidence stream contributing to the overall evaluation. From the perspective of medical education, these two preambles lay the foundation for the epidemiological evaluation of the available scientific evidence.

The first chapter details the anatomy of the oral cavity and the oropharynx, followed by an overview of oral cancer, oropharyngeal cancer and oral potentially malignant disorders (OPMDs). Further discussion focuses on the natural history, the stage at diagnosis, the survival rates, and the available treatment and management options for oral cancer and OPMDs.

**Cite as**
Duś-Ilnicka I. Neglected area of oral cancer: A word about the International Agency for Research on Cancer (IARC) "Handbook of Oral Cancer Prevention". *Dent Med Probl.* 2024;61(4):479–480. doi:10.17219/dmp/190468

**DOI**
10.17219/dmp/190468

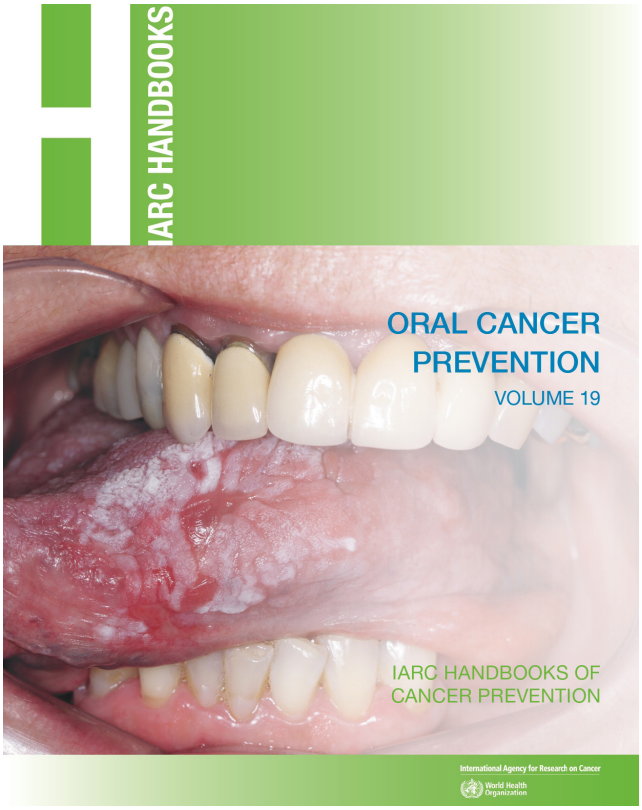


Fig. 1. International Agency for Research on Cancer (IARC) "Handbook of Oral Cancer Prevention", vol. 19 (title page)

The second chapter begins with the description of the established risk factors for oral cancer. Then, it evaluates the evidence regarding the impact of quitting tobacco smoking, reducing alcohol consumption, stopping smokeless tobacco (SLT) use, and refraining from chewing areca nut products (including betel quid) with and without tobacco on reducing oral cancer development. Additionally, this chapter presents the evidence on the association between the consumption of preventive dietary agents, such as coffee, tea, fruits, vegetables, and dietary fiber, on oral cancer and OPMD development.

The third chapter depicts the various types of SLT and areca nut products used worldwide, providing a detailed description of their prevalence across different World Health Organization (WHO) regions. The chapter also reviews the available evidence on various interventions (behavioral, pharmacological – alone or in combination) for the cessation of using SLT or areca nut products among both adults and adolescents. In the context of global health discussion, the comprehensive description of this topic in Handbook 19 can help broaden the knowledge about SLT use, even in countries where areca nut products or SLT are less prevalent, but where international students are taught about oral cancer epidemiology and prevention.

The fourth chapter discusses the available methods for screening and the early diagnosis of oral cancer. Correct clinical oral examination requires expertise and training in screening for oral mucosal abnormalities (OPMDs and oral cancer). The chapter also explores innovative approaches, like mobile phone applications and patient self-examination for oral cancer screening. Additionally, adjunctive methods, such as autofluorescence, narrow-band imaging, tissue reflectance, and vital staining, are described. The second half of the chapter discusses the existing organized and opportunistic screening activities, as well as the factors influencing participation, and presents a systematic review of the evidence on the benefits and risks of screening for oral cancer.

Following the four main chapters, the fifth chapter offers the summaries of the preceding text. The sixth chapter, "Evaluations, Statements, and Considerations", presents the evaluations made by the working group and the rationale behind these assessments.[2]

A special report detailing the evaluations of this handbook has also been published.[2]

In conclusion, Handbook 19 provides a thorough and comprehensive evidence-based evaluation of primary and secondary prevention interventions for oral cancer, including cessation strategies for SLT and areca nut products, the impact of reducing exposure to risk factors, and the effectiveness of screening for oral cancer. The information contained in the handbook could be a useful addition to dental school curricula, particularly in shaping subjects such as oral pathology and oral medicine, as well as in promoting the dissemination of knowledge on oral cancer prevention and screening.

## ORCID iDs

Irena Duś-Ilnicka https://orcid.org/0000-0002-4745-2560

## References

1. World Helath Organization (WHO), International Agency for Research on Cancer (IARC). *IARC Handbook of Oral Cancer Prevention*. Vol. 19. Geneva, Switzerland: WHO, IARC; 2023.
2. Bouvard V, Nethan ST, Singh D, et al. IARC perspective on oral cancer prevention. *N Engl J Med*. 2022;387(21):1999–2005. doi:10.1056/NEJMsr2210097

# Removal of mandibular third molars: An overview of risks, a proposal for international community and guidance

Mohammed Murad[1,2,A–F], Lolwa Al-Maslamani[3,E,F], Julian Yates[4,A–F]

[1] Division of Dentistry, Primary Health Care Corporation, Doha, Qatar
[2] Division of Dentistry, College of Dental Medicine, Qatar University, Doha, Qatar
[3] Division of Dentistry, Hamad Medical Corporation, Doha, Qatar
[4] Division of Dentistry, School of Medical Sciences, University of Manchester, UK

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*. 2024;61(4):481–488

**Address for correspondence**
Mohammed Murad
E-mail: dr_m_murad@hotmail.com

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on March 16, 2023
Reviewed on May 9, 2023
Accepted on May 15, 2023

Published online on August 14, 2024

**Keywords:** prophylactic removal, third molar surgery, dental extraction, impacted third molars, impacted wisdom teeth

**The aim of this proposal is to (1) review the current literature, (2) shed light on the importance of creating universally accepted guidelines, (3) provide help and guidance in the decision-making process with regard to the removal of mandibular third molars (M3Ms), and (4) reduce the risk of exposing the patient to unnecessary harm and complications due to the inappropriate removal or retention of M3Ms.**

**It is obvious that the indications for the extraction of M3Ms will continue to be an area of controversy and strong debate. The evidence for or against prophylactic extraction is ambivalent; there is evidence to accept or reject the stance against prophylactic extraction in some specific cases, and there are published articles to support both opposing views. The available guidelines on the extraction of third molars are limited in number, and are mostly tailored to fit specific settings or countries. There are no available guidelines that might be widely used to help in the decision-making process for the international community. We hope this proposal will constitute an important first step toward creating universally accepted guidance.**

**Cite as**
Murad M, Al-Maslamani L, Yates J. Removal of mandibular third molars: An overview of risks, a proposal for international community and guidance. *Dent Med Probl.* 2024;61(4):481–488. doi:10.17219/dmp/166156

**DOI**
10.17219/dmp/166156



## Introduction

Third molars usually erupt at the age of 17–26 years,[1–3] and can cause significant problems both in the short and long term. The eruption process can generally follow one of 2 distinct patterns – impacted (vertical, mesial, distal, horizontal, or transverse) or non-impacted (fully erupted). Although both impacted and non-impacted mandibular third molars (M3Ms) can be asymptomatic and disease-free, their eruption can sometimes be associated with significant pain, inflammation, swelling, difficulties in mastication, limited mouth opening, and/or acute pericoronitis. In some cases, these pathologies may be significant enough to cause considerable discomfort and facial swelling, thereby contributing to morbidity in the affected individuals.

Furthermore, both impacted and non-impacted M3Ms can be associated with dental-related pathologies, such as tumors, cysts, caries, and/or periodontal disease, which may not be limited to third molars, but also affect the adjacent tissues and teeth. All of these factors may indicate the need for the removal of M3Ms.[4]

Some clinicians and patients have long held the notion that M3Ms may be prophylactically removed to avoid the potential pathologies arising from their impaction. However, many healthcare systems do not agree with the assertion that lower third molars should be removed in the absence of an overt pathology or other clinical justification.[5]

Currently, there is limited data available to estimate how many healthy and asymptomatic M3Ms have been removed without justification. However, it is believed that in the past, up to 44% of healthy wisdom teeth were inappropriately removed in the UK.[6]

There are varying approaches to the management of M3Ms, leading to the creation of clinical guidelines in this regard, especially in the UK. However, these and similar clinical guidelines are often criticized, amended, or even withdrawn due to the lack of consensus on their appropriateness. Despite these varied recommendations, the prophylactic removal of M3Ms is still routinely undertaken and accepted in many parts of the world.[7] Given these diverse recommendations and concerns about the management of M3Ms, an evidence-based approach that would benefit clinicians and patients alike is highly desirable. The management of M3Ms may remain controversial with differing opinions, but the aim of this proposal is to emphasize the importance of creating universally accepted guidelines. We hope this proposal will constitute an important first step toward creating such guidelines.

## Methodology

While conducting the review, the following databases were electronically searched up to January 2023: MEDLINE; the Cochrane Central Register of Controlled Trials (CENTRAL); PubMed; Google Scholar; and the Cochrane Library. A hand search of the reference lists of the included articles was also conducted. The search was restricted to articles published in English. After removing duplicates, the search yielded 486 articles, but only 12 guidelines and position statements were identified.

## Available guidelines

The M3M is an unpredictable tooth in terms of development and clinical presentation. As such, it is not surprising that the available guidelines on its management are limited in number. In the UK, the National Institute for Health and Care Excellence (NICE) released clinical guidance in 2000, recommending that asymptomatic impacted M3Ms should not be removed, and only those with a defined pathology should be considered.[6] The Scottish Intercollegiate Guidelines Network (SIGN) also issued guidance on the management of impacted, unerupted M3Ms; however, the guidance was withdrawn in 2015.[8]

In the USA, in 2016, the American Association of Oral and Maxillofacial Surgeons (AAOMS) issued a white paper on the management of M3Ms.[9] Aside from this, there is very little guidance comparable to that released in the UK. The paper is a position statement on the management of third molars rather than actual guidelines. The white paper strongly recommends thorough evaluation by a surgeon, with the trend in the USA leaning toward removing the third molar in young individuals, even if the tooth is healthy and asymptomatic.[9] Consequently, the position statement from AAOMS has not been without criticism.[6,10,11]

In 2014, the Finnish Medical Society Duodecim published guidelines recommending the prophylactic removal of asymptomatic and disease-free M3Ms in specific situations to avoid possible future complications, such as injury to the inferior alveolar nerve, pericoronitis, postoperative bone defects, visible plaque in the adjacent teeth, or dental caries.[7] However, there are 2 contraindications to such intervention: 1) if the asymptomatic unerupted M3M is healthy and completely covered by bone; and 2) if the removal could pose a significant or unreasonable risk to the patient's health.[7]

Given the contrasting opinions on the subject, a Cochrane review aimed to clarify the evidence on the prophylactic removal of asymptomatic impacted wisdom teeth vs. the retention of the tooth.[3] The review concluded that the evidence either supporting or refuting the routine prophylactic removal of asymptomatic healthy impacted third molars was insufficient.[3]

It is apparent that the management of impacted M3Ms varies considerably, despite all the available guidelines being based on the same available evidence. These differences seem to be influenced by several factors, including resources, environment, surgical trends, culture, reimbursement by insurance companies, and government actions.[7] For instance, in the UK, the surgical removal of impacted third molar teeth is funded by the National Health Service (NHS). In 1990, approx. £30 million was spent by NHS on this procedure; similarly, in the private sector, approx. £22 million was spent in the same year.[12–14] Therefore, it is suggested that the prophylactic removal of M3Ms contributes significantly to increased healthcare costs that could potentially be utilized for other healthcare needs. The potential cost savings could be considerable, since there is no evidence that retaining healthy and impacted M3Ms leads to unmanageable symptoms, pathologies or serious consequences for the patient.[3]

Given the issues related to this topic, it is important to use an evidence-based approach to evaluate the risks inherent in the retention vs. removal of impacted lower third molar teeth. The number of voices highlighting the inadequacy of the current guidelines is increasing, and it is increasingly acknowledged by clinicians that in some cases, the development and progression of symptoms and pathologies can be largely predicted, and that surgery to remove M3Ms in older patients can lead to complications and patient morbidity.[15–18]

## Risks associated with retention

A study conducted at Guy's Hospital, London, UK, investigated the clinical records of 100 patients who had 122 M3Ms removed due to distal cervical caries in the adjacent second molar.[19] The study reported that the early or prophylactic extraction of a partially erupted third molar could have prevented the formation of distal cervical caries in a mandibular second molar. This suggestion was specifically related to M3Ms that were partially erupted and mesially impacted between 40° and 80° in relation to the second molar.[19]

In 2014, a follow-up study confirmed that distal cervical caries in mandibular second molars is a late complication of third molar retention.[20] The study also confirmed that the prophylactic removal of partially erupted mesioangular M3Ms could prevent this complication.[20] In 2013, Nunn et al. reported a 2.5-fold increase in the rate of distal caries in second molars adjacent to erupted third molars.[21] These findings were consistent with those reported by Pepper et al. in 2017, who found a 7% increase in distal caries of a second molar when associated with a partially erupted third molar.[22]

In 2016, Toedtling et al. suggested the following indicators of the development of distal cervical caries in mandibular second molars: 1) the eruption status of the M3M, with longer periods of partial eruption increasing the risk of distal cervical caries; 2) the type of angulation, with mesioangular and horizontal impaction associated with 85% of the carious second molars observed; and 3) the position of the mesial cusps of the M3M in relation to the amelo-cemental junction (ACJ) of the second molar, with distal caries being notably higher when mesioangular impaction is below ACJ.[17]

A more recent systematic review with medium- to fair-quality evidence concluded that retained asymptomatic third molars, especially when partially erupted, were more likely to develop a disease due to impaction, age and the retention time.[16] Furthermore, retention significantly increases the risk of pathology in a second molar, especially when associated with partially or mesially erupted M3Ms.[16]

Another study examined the impact of the NICE guidelines on the removal of M3Ms.[5] The study indicated a shift in M3M removal from a young to an older adult population, with the mean age of patients undergoing treatment increasing from 25 to 32 years. However, it is important to note that age itself is not a causal factor for increased complication rates; rather, aging patients tend to have more complex health conditions that may affect their ability to cope with or recover from such procedures. The study also noted that the number of patients requiring M3M removal remained comparable to that in the mid-1990s, suggesting that the NICE guidelines did not reduce the need for treatment. However, the potential unintentional biases in the study, such as changes in the UK population between the 1990s and 2012, were acknowledged. It was unclear whether this population increase was accounted for in the study. The study also indicated discrepancies in treatment coding, data collection and the inclusion of outpatients as compared to data from the 1990s.[5]

Periodontal disease associated with M3Ms has also been suggested as an indication for their removal. Increased periodontal probing depths are often associated with retained M3Ms, which have been reported to serve as a reservoir harboring microorganisms linked to periodontal disease. The early removal of M3Ms may prevent the onset and progression of periodontal disease.[23] However, integrating periodontal assessment into the regular monitoring and follow-up of third molars may be a more suitable approach. In a prospective cohort study involving 804 third molars with a follow-up period ranging from 3 to over 25 years, limited yet credible evidence suggested that the presence of healthy, symptom-free impacted third molars might increase the risk of periodontal disease around the adjacent teeth.[3,21] On the other hand, the surgical removal of impacted wisdom teeth has been associated with the loss of periodontal attachment on the distal part of the adjacent tooth.[24]

## Risks associated with removal

The extraction of M3Ms is one of the most common routine procedures in oral surgery. A retrospective cohort study examined 583 patients with 1,597 third molars, finding an overall complication rate of 4.6%; the most frequent complications were alveolitis, delayed healing and damage to the inferior alveolar nerve.[25]

However, another prospective study assessed surgical and post-surgical complications following the extraction of M3Ms in 9,574 patients with 16,127 M3Ms removed.[26] This review concluded an overall complication rate of 10.8%. Common potential complications included pain, bleeding, infection, local swelling, damage to the adjacent anatomical structures (including tooth and periodontal defects), alveolar osteitis, restricted mouth opening, incomplete root removal, temporomandibular joint (TMJ) injury, displacement, lingual nerve damage, inferior alveolar nerve damage, and jaw fracture.[25–28] It is acknowledged that most of these are considered the side effects of surgery rather than adverse complications.

## Paresthesia

Damage to the lingual nerve and/or the inferior alveolar nerve constitutes the most serious of all the most common complications, and leads to the most frequent malpractice and negligence claims.

A retrospective study on the incidence of inferior alveolar nerve paresthesia after the surgical extraction of M3Ms included 3,286 patients.[29] The study concluded that the prevalence of paresthesia as a complication of surgery was 1.5% at 1 month post procedure. However, this incidence was further reduced to only 0.6% 2 years after the procedure.[29] Another study reported that in most cases, injury due to the removal of M3Ms had healed within 1–2 months after the procedure.[30] A systematic review also determined that the rate of paresthesia of the inferior alveolar nerve was between 0.35% and 8.40%.[31]

Injury to the lingual nerve is not a common complication of the procedure. Nevertheless, it can be a significant and troubling complication when it does occur. A study that referred to 1,117 procedures found that the rate of injury to the lingual nerve was approx. 11%.[32] However, full recovery from injury could take up to 9 months, with approx. 50% of patients having fully recovered within 6 weeks of the procedure. Importantly, a number of these patients (0.5%) had permanent injury to the lingual nerve and will not make full recovery.[32] In another review reporting on outcomes following M3M removal, the prevalence of temporary injury was estimated between 0% and 37%, whereas the prevalence of permanent injury was estimated between 0% and 2%.[33] Moreover, when injuries to the lingual and inferior alveolar nerves in patients are considered together, the incidence ranges from 0% to 15%.[25,34–36]

These injuries continue to be a contentious point for both the patient and the surgeon despite their relatively low rates, especially if followed by legal disputes that become protracted and time-consuming. These injuries, especially inferior alveolar nerve and lingual nerve injuries, can lead to profoundly detrimental physical and psychological effects on the affected patients, and psychological effects on the surgeon.

## Alveolar osteitis (dry socket)

Alveolar osteitis is the most common temporary complication associated with the extraction of M3Ms. Although it is not a significant concern clinically and it is relatively straightforward to manage, it can be burdensome to the patient due to the severe pain associated with the condition. It usually starts 3–4 days after surgery, radiates from the site of extraction, and is commonly associated with a foul odor and/or a bad taste. The exact cause of dry socket is still unknown, but it seems to be linked to several contributing factors, including the patient's age, medical and dental status, smoking, patient compliance with hygiene instructions, and the complexity of the surgical procedure itself.[37]

## Infection

Most infections associated with the removal of M3Ms can be readily managed, as, on the whole, they are self-limiting or only require local measures to resolve. However, some patients may require systemic antimicrobial therapy, or even extended hospitalization and intravenous antibiotics due to the development and progression of serious infections. A small number of patients may require further surgical intervention to treat such infections. These infections also have the potential to become systemic infections, leading to sepsis, septic shock and, rarely, life-threatening complications.[38]

The rate of postoperative local infections associated with the surgical extraction of M3Ms is between 0.8% and 4.2%.[27] The incidence of systemic infections remains low, although the exact number is uncertain due to limited studies on the topic. A Japanese study reported a rate of deep fascial space infections after the surgical removal of M3Ms at 0.8%.[39] In another study investigating 723 cases over 1 year, the rate of systemic infections involving deep fascial spaces in the neck was 0.5%.[38] These patients required hospitalization, and both medical and surgical treatment.[38] This highlights that while severe deep fascial space infections are rare, they are significant complications following extraction and can pose a threat to life.

## Fracture

Mandibular fracture is a serious complication associated with M3M removal, but it is extremely rare, reported in approx. 0.0049% of cases.[40] Although troublesome in its own right, this can also lead to litigation, especially if the outcome was not anticipated or if the fracture was associated with nerve injury.

## Bleeding

Most reported intra- and postoperative bleeding can be adequately controlled with local measures. However, there are cases of excessive bleeding associated with M3M extraction, reported to range between 0.2% and 58.0%.[27] Excessive bleeding can lead to the formation of hematomata, the stiffness of the mastication muscles, trismus and, in severe cases, airway obstruction and significant patient morbidity. The most worrisome cases of excessive bleeding are those potentially associated with patients who have bleeding disorders that have not been previously diagnosed, such as hemophilia A or B, von Willebrand disease, and mandibular artero-venous malformations.[26] Unfortunately, some disorders may not be suspected until

problems arise during or after surgery, as the removal of M3Ms may be the first surgical intervention many patients encounter.[28,41–43]

### Displacement

The displacement of third molars, third molar roots or third molar fragments is another rare complication that can lead to serious issues. In a review by Di Nardo et al., it was noted that the most frequently reported site for mandibular displacement is the submandibular tissue space.[44] Other reported sites include the sublingual space, the pterygomandibular fossa, the buccal space, the lateral cervical space, the lateral pharyngeal space, and the body/ramus of the mandible.[25,27,28,45] Depending on the location of displacement and proximity to vital structures, attempts at retrieval could complicate matters and necessitate unintended surgical procedures.

### Other complications

There are several other potential complications associated with the removal of M3Ms. However, a detailed discussion of self-limiting complications that are easily managed is beyond the scope of this paper.

## Introduction to the proposal

The available guidelines on the extraction of third molars are limited in number, and are mostly tailored to specific settings or countries. However, there are no widely used guidelines available to assist the international community in the decision-making process. The purpose of this proposal is to provide further details about the existing guidelines and recommendations. This proposal is primarily based on the published guidance, with amendments to include the most appropriate and salient points from each. This proposal could potentially be used and further developed by the international community to establish consensus, and assist both patients and clinicians alike. More specifically, this protocol should be considered as a guide only to help in the decision-making process.

Since the future of such unpredictable teeth cannot be accurately anticipated, clinical judgment should always be exercised. Cases should be evaluated on a case-by-case basis, with the final decision based on a joint agreement between the patient and the clinician.[9]

All patients should be fully informed about the advantages, disadvantages, risks, benefits, and the potential complications associated with the removal or retention of M3Ms.

Patients should be actively involved in the decision-making process.

## Purpose of the proposal

The purpose of the current proposal is:
– to prevent the inappropriate prophylactic removal of M3Ms;
– to prevent the inappropriate retention of M3Ms;
– to provide help and guidance in the decision-making process regarding M3M removal; and
– to reduce the risk of exposing the patient to unnecessary harm and complications due to the inappropriate removal or retention of M3Ms.

## Proposal

Pathology-free impacted M3Ms should not be removed[6] unless they are at a significant risk of developing a disease[7,9] that cannot be conservatively managed or treated.

The prophylactic extraction of healthy M3Ms should be discontinued[6] unless mandibular second molars are at a significant risk of developing a disease[7] that cannot be conservatively managed or treated.

The first episode of pericoronitis should be treated conservatively and should not be considered as an indication for surgery. Depending on their severity, recurrent episodes of pericoronitis may be considered an indication for surgery.[6]

The absence of symptoms does not indicate the absence of a disease. Therefore, regular follow-up, clinical assessment and/or radiographic surveillance are advisable.[9]

## Pathologies or conditions that may justify surgical intervention

The following pathologies or conditions may justify surgical intervention:
– non-restorable wisdom teeth and/or untreatable tooth decay, and pulpal and/or periapical pathology[6];
– abscesses, cellulitis and/or osteomyelitis[6];
– tumors or cysts[6];
– pathologies of the tooth and/or the adjacent structures that cannot be treated conservatively[6];
– the tooth obstructs other surgery/treatment or its removal is indicated for other surgery/treatment[6,9];
– the associated tissue requires histological examination[9]; and
– the overlying removable prostheses.[9]

The management flowchart for M3Ms is presented in Fig. 1.

## Other considerations

Generally speaking, there is agreement that diseased, symptomatic and non-restorable M3Ms should be removed. However, for healthy, asymptomatic and restorable M3Ms, there is less consensus (Fig. 2).[9]

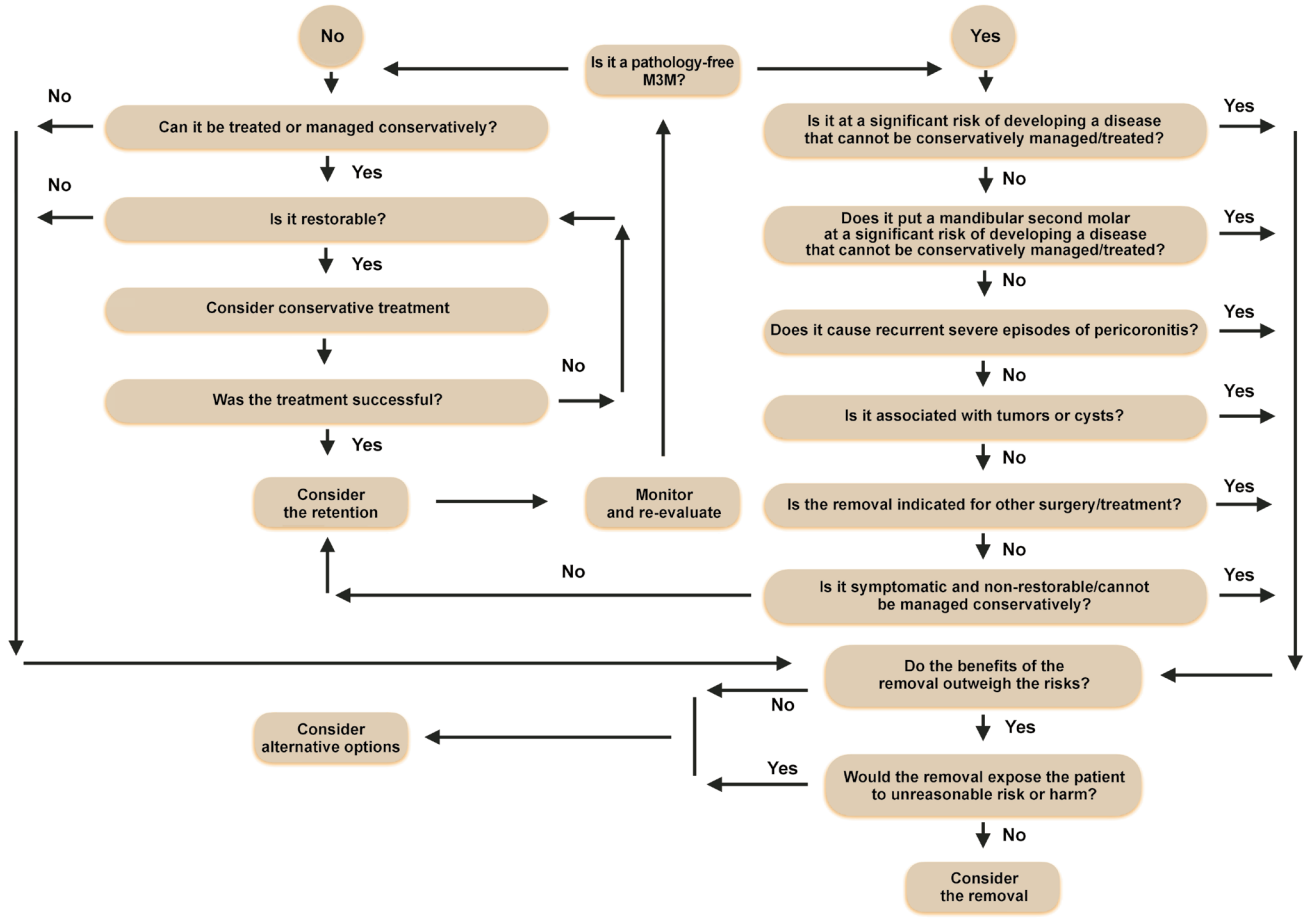


Fig. 1. Management flowchart for mandibular third molars (M3Ms)

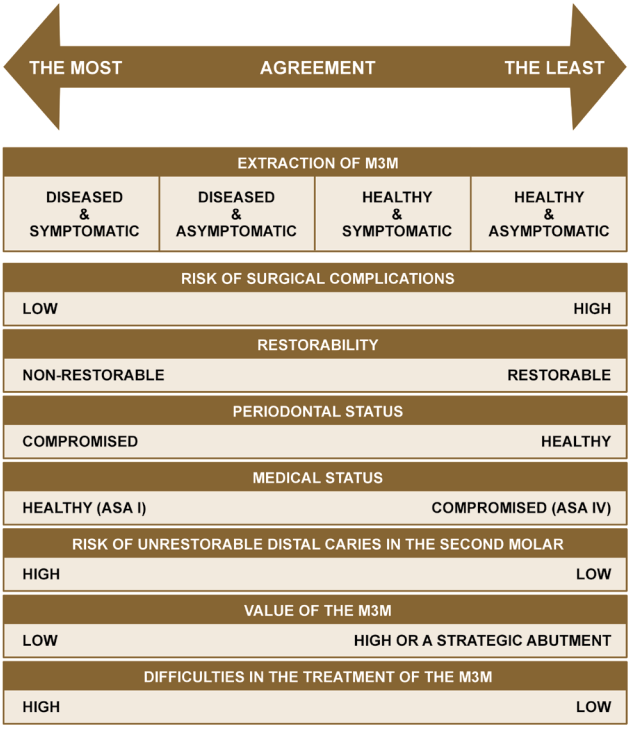


Fig. 2. Agreement chart with regard to the extraction of mandibular third molars (M3Ms)

ASA – the American Society of Anesthesiologists (ASA) physical status classification system.

The most frequent dilemmas faced by clinicians regarding M3Ms include the following:

- the risks and benefits associated with the surgical removal of M3Ms[9];
- the risks and benefits associated with the retention of M3Ms[9];
- the risks and benefits associated with the partial removal (coronectomy) of M3Ms[9];
- the risks and benefits associated with the surgical exposure of M3Ms[9];
- the most appropriate timing for treatment[9];
- the patient's age and the likelihood of developing associated diseases[6,7,9];
- the current symptoms[6,9];
- the position, angulation, eruption, and anatomy of the M3M[7];
- the functional, periodontal and carious status of the M3M[6,7,9];
- the feasibility of other treatment and the restorability of the tooth[6,7,9];
- the cost associated with the surgical removal of M3Ms[9];
- the cost associated with the retention of M3Ms[9]; and
- the most appropriate plan for follow-up, clinical and radiographic examinations.[9]

## Contraindications for the removal of M3Ms

Below are presented the contraindications for the removal of M3Ms:

– the removal would expose the patient to unreasonable risk or harm[7];
– unerupted, healthy and asymptomatic M3Ms that are fully covered by bone, with no potential risk of developing a disease[7]; and
– healthy and asymptomatic M3Ms that are at a minimal risk of developing a disease and can be treated conservatively.[9]

## Conclusions

It is obvious that the indications for the extraction of M3Ms will continue to be an area of controversy and strong debate. The evidence for or against prophylactic extraction is ambivalent; there is evidence to accept or reject the stance against prophylactic extraction in some specific cases, and there are published articles to support both opposing views.

In the UK, NICE stated that "there is no reliable research evidence to support a health benefit to patients from the prophylactic removal of pathology-free impacted third molar teeth".[6] The AAOMS white paper stated that "uncertainty is more explicit in the case of patients who have asymptomatic, disease-free third molars",[9] while the Finnish guidelines stated that "patients would benefit markedly from elective preventive removal of third molars".[7]

In 2016, a study undertaken by Cochrane reviewed the prophylactic removal of asymptomatic impacted wisdom teeth against their retention, and concluded that "insufficient evidence is available to determine whether or not asymptomatic, disease-free impacted wisdom teeth should be removed".[3]

The unpredictable nature of M3Ms will continue to cause controversy, especially in providing universally accepted guidelines for their removal. It is acknowledged that in the vast majority of cases, their removal goes without incident, but in a small but significant number of patients, the sequelae of removal can have a profound negative impact on the patients and their quality of life. However, in recent years, there has been growing evidence that the retention of asymptomatic and disease-free M3Ms can also lead to adverse outcomes, including the loss of the adjacent second molars, many of which could have been prevented if removal had been undertaken earlier.

Therefore, while it may be wise to take extra caution when considering the prophylactic removal of such an unpredictable tooth, anticipating which teeth may cause problems in the future may help influence our decision-making process for the long-term benefit of both the patient and the clinician.

### ORCID iDs

Mohammed Murad https://orcid.org/0000-0001-8546-2121
Lolwa Al-Maslamani https://orcid.org/0009-0009-5427-158X
Julian Yates https://orcid.org/0000-0002-8187-023X

### References

1. De Bruyn L, Vranckx M, Jacobs R, Politis C. A retrospective cohort study on reasons to retain third molars. *Int J Oral Maxillofac Surg*. 2020 Jun;49(6):816–821. doi:10.1016/j.ijom.2019.10.003
2. Renton T, Wilson NH. Problems with erupting wisdom teeth: Signs, symptoms, and management. *Br J Gen Pract*. 2016;66(649):e606–e608. doi:10.3399/bjgp16X686509
3. Ghaeminia H, Perry J, Nienhuijs ME, et al. Surgical removal versus retention for the management of asymptomatic disease-free impacted wisdom teeth. *Cochrane Database Syst Rev*. 2016;8:CD003879. doi:10.1002/14651858.CD003879.pub4
4. McArdle LW, Andiappan M, Khan I, Jones J, McDonald F. Diseases associated with mandibular third molar teeth. *Br Dent J*. 2018;224(6):434–440. doi:10.1038/sj.bdj.2018.216
5. McArdle LW, Renton T. The effects of NICE guidelines on the management of third molar teeth. *Br Dent J*. 2012;213(5):E8. doi:10.1038/sj.bdj.2012.780
6. National Institute for Health and Care Excellence (NICE). Guidance on the extraction of wisdom teeth. https://www.nice.org.uk/guidance/ta1/resources/guidance-on-the-extraction-of-wisdom-teeth-pdf-63732983749. Accessed May 21, 2020.
7. Ventä I. Current care guidelines for third molar teeth. *J Oral Maxillofac Surg*. 2015;73(5):804–805. doi:10.1016/j.joms.2014.12.039
8. Scottish Intercollegiate Guidelines Network (SIGN). Management of unerupted and impacted third molar teeth – SIGN 43. https://www.scottishdental.nhs.scot/library/management-of-unerupted-and-impacted-third-molar-teeth. Accessed May 23, 2020.
9. American Association of Oral and Maxillofacial Surgeons (AAOMS). Management of third molar teeth. https://www.aaoms.org/docs/govt_affairs/advocacy_white_papers/management_third_molar_white_paper.pdf. Accessed May 24, 2020.
10. Friedman JW. The prophylactic extraction of third molars: A public health hazard. *Am J Public Health*. 2007;97(9):1554–1559. doi:10.2105/AJPH.2006.100271
11. Mansoor J, Jowett A, Coulthard P. NICE or not so NICE? *Br Dent J*. 2013;215(5):209–212. doi:10.1038/sj.bdj.2013.832
12. Landes DP. The relationship between dental health and variations in the level of third molar removals experienced by populations. *Community Dent Health*. 1998;15(2):67–71. PMID:9793220.
13. Thomas D, Walker R, Smith A, Shepherd J. The provision of oral surgery services in England and Wales 1984–1991. *Br Dent J*. 1994;176(6):215–219. doi:10.1038/sj.bdj.4808417
14. Fernandes MJ, Ogden GR, Pitts NB, Ogston SA, Ruta DA. Incidence of symptoms in previously symptom-free impacted lower third molars assessed in general dental practice. *Br Dent J*. 2009;207(5):E10. doi:10.1038/sj.bdj.2009.804
15. Vranckx M, Fieuws S, Jacobs R, Politis C. Prophylactic vs. symptomatic third molar removal: Effects on patient postoperative morbidity. *J Evid Based Dent Pract*. 2021;21(3):101582. doi:10.1016/j.jebdp.2021.101582
16. Vandeplas C, Vranckx M, Hekner D, Politis C, Jacobs R. Does retaining third molars result in the development of pathology over time? A systematic review. *J Oral Maxillofac Surg*. 2020;78(11):1892–1908. doi:10.1016/j.joms.2020.06.014
17. Toedtling V, Coulthard P, Thackray G. Distal caries of the second molar in the presence of a mandibular third molar – a prevention protocol. *Br Dent J*. 2016;221(6):297–302. doi:10.1038/sj.bdj.2016.677
18. Jaroń A, Trybek G. The pattern of mandibular third molar impaction and assessment of surgery difficulty: A retrospective study of radiographs in East Baltic population. *Int J Environ Res Public Health*. 2021;18(11):6016. doi:10.3390/ijerph18116016
19. McArdle LW, Renton TF. Distal cervical caries in the mandibular second molar: An indication for the prophylactic removal of the third molar? *Br J Oral Maxillofac Surg*. 2006;44(1):42–45. doi:10.1016/j.bjoms.2005.07.025

20. McArdle LW, McDonald F, Jones J. Distal cervical caries in the mandibular second molar: An indication for the prophylactic removal of third molar teeth? Update. *Br J Oral Maxillofac Surg*. 2014;52(2):185–189. doi:10.1016/j.bjoms.2013.11.007
21. Nunn ME, Fish MD, Garcia RI, et al. Retained asymptomatic third molars and risk for second molar pathology. *J Dent Res*. 2013;92(12):1095–1099. doi:10.1177/0022034513509281
22. Pepper T, Grimshaw P, Konarzewski T, Combes J. Retrospective analysis of the prevalence and incidence of caries in the distal surface of mandibular second molars in British military personnel. *Br J Oral Maxillofac Surg*. 2017;55(2):160–163. doi:10.1016/j.bjoms.2016.10.009
23. Kindler S, Holtfreter B, Koppe T, et al. Third molars and periodontal damage of second molars in the general population. *J Clin Periodontol*. 2018;45(11):1365–1374. doi:10.1111/jcpe.13008
24. Aniko-Włodarczyk M, Jaroń A, Preuss O, Grzywacz A, Trybek G. Evaluation of the effect of surgical extraction of an impacted mandibular third molar on the periodontal status of the second molar – prospective study. *J Clin Med*. 2021;10(12):2655. doi:10.3390/jcm10122655
25. Bui CH, Seldin EB, Dodson TB. Types, frequencies, and risk factors for complications after third molar extraction. *J Oral Maxillofac Surg*. 2003;61(12):1379–1389. doi:10.1016/j.joms.2003.04.001
26. Osborn TP, Frederickson G Jr., Small IA, Torgerson TS. A prospective study of complications related to mandibular third molar surgery. *J Oral Maxillofac Surg*. 1985;43(10):767–769. doi:10.1016/0278-2391(85)90331-3
27. Bouloux GF, Steed MB, Perciaccante VJ. Complications of third molar surgery. *Oral Maxillofac Surg Clin North Am*. 2007;19(1):117–128, vii. doi:10.1016/j.coms.2006.11.013
28. Wayland J. *Impacted Third Molars*. Hoboken, NJ: John Wiley & Sons, Inc.; 2018:33–65.
29. Jerjes W, Upile T, Shah P, et al. Risk factors associated with injury to the inferior alveolar and lingual nerves following third molar surgery – revisited. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2010;109(3):335–345. doi:10.1016/j.tripleo.2009.10.010
30. Alling CC 3rd. Dysesthesia of the lingual and inferior alveolar nerves following third molar surgery. *J Oral Maxillofac Surg*. 1986;44(6):454–457. doi:10.1016/s0278-2391(86)80010-6
31. Sarikov R, Juodzbalys G. Inferior alveolar nerve injury after mandibular third molar extraction: A literature review. *J Oral Maxillofac Res*. 2014;5(4):e1. doi:10.5037/jomr.2014.5401
32. Blackburn CW, Bramley PA. Lingual nerve damage associated with the removal of lower third molars. *Br Dent J*. 1989;167(3):103–107. doi:10.1038/sj.bdj.4806922
33. Pippi R, Spota A, Santoro M. Prevention of lingual nerve injury in third molar surgery: Literature review. *J Oral Maxillofac Surg*. 2017;75(5):890–900. doi:10.1016/j.joms.2016.12.040
34. Gargallo-Albiol J, Buenechea-Imaz R, Gay-Escoda C. Lingual nerve protection during surgical removal of lower third molars. A prospective randomised study. *Int J Oral Maxillofac Surg*. 2000;29(4):268–271. PMID:11030397.
35. Cheung LK, Leung YY, Chow LK, Wong MCM, Chan EKK, Fok YH. Incidence of neurosensory deficits and recovery after lower third molar surgery: A prospective clinical study of 4338 cases. *Int J Oral Maxillofac Surg*. 2010;39(4):320–326. doi:10.1016/j.ijom.2009.11.010
36. Bataineh AB. Sensory nerve impairment following mandibular third molar surgery. *J Oral Maxillofac Surg*. 2001;59(9):1012–1017. doi:10.1053/joms.2001.25827
37. Kolokythas A, Olech E, Miloro M. Alveolar osteitis: A comprehensive review of concepts and controversies. *Int J Dent*. 2010;2010:249073. doi:10.1155/2010/249073
38. O'Connor N, Besi E, Lopes V, Malden N. Incidence of deep fascial space infections following lower third molar removal. *Oral Surg*. 2017;11(1):17–21. doi:10.1111/ors.12278
39. Yoshii T, Hamamoto Y, Muraoka S, et al. Incidence of deep fascial space infection after surgical removal of the mandibular third molars. *J Infect Chemother*. 2001;7(1):55–57. doi:10.1007/s101560170036
40. Libersa P, Roze D, Cachart T, Libersa JC. Immediate and late mandibular fractures after third molar removal. *J Oral Maxillofac Surg*. 2002;60(2):163–165. doi:10.1053/joms.2002.29811
41. Rafique S, Fiske J, Palmer G, Daly B. Special care dentistry: Part 1. Dental management of patients with inherited bleeding disorders. *Dent Update*. 2013;40(8):613–616,619–622,625–626 passim. doi:10.12968/denu.2013.40.8.613
42. Baghaie H, Forrest B, Shukla K, Liu T. Dental extraction in a patient with undiagnosed von Willebrand's disease: A case report. *Aust Dent J*. 2021;66(1):105–111. doi:10.1111/adj.12788
43. Orlian AI, Karmel R. Postoperative bleeding in an undiagnosed hemophilia A patient: Report of case. *J Am Dent Assoc*. 1989;118(5):583–584. doi:10.14219/jada.archive.1989.0083
44. Di Nardo D, Mazzucchi G, Lollobrigida M, et al. Immediate or delayed retrieval of the displaced third molar: A review. *J Clin Exp Dent*. 2019;11(1):e55–e61. doi:10.4317/jced.55379
45. Huang IY, Wu CW, Worthington P. The displaced lower third molar: A literature review and suggestions for management. *J Oral Maxillofac Surg*. 2007;65(6):1186–1190. doi:10.1016/j.joms.2006.11.031

Original paper

# Prevalence of cranial autonomic symptoms in frequent episodic tension-type headache: A post hoc analysis of the cross-sectional Migraine in Poland study

Marcin Straburzyński[1,A–D,F], Marta Waliszewska-Prosół[2,A–C,E,F], Magdalena Nowaczewska[3,B,E,F], Ewa Katarzyna Czapinska-Ciepiela[4,B,E,F], Anna Gryglas-Dworak[5,B,E,F], Slawomir Budrewicz[2,A,E,F]

[1] Department of Family Medicine and Infectious Diseases, University of Warmia and Mazury, Olsztyn, Poland
[2] Department of Neurology, Wroclaw Medical University, Poland
[3] Department of Otolaryngology, Head and Neck Surgery and Laryngological Oncology, Ludwik Rydygier Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Bydgoszcz, Poland
[4] Epilepsy and Migraine Treatment Centre, Cracow, Poland
[5] MIGRE Polish Migraine Center, Wrocław, 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*. 2024;61(4):489–493

**Address for correspondence**
Marcin Straburzyński
E-mail: marcin.straburzynski@uwm.edu.pl

**Funding sources**
None declared

**Conflict of interest**
In the past 24 months, Marcin Straburzyński, PhD, Magdalena Nowaczewska, PhD, and Ewa Katarzyna Czapinska-Ciepiela, PhD, have received honoraria for lectures (Pfizer, AbbVie, Bausch Health Poland, Teva; Pfizer, Teva; Teva, Angelini Pharma Polska, Pfizer, respectively) and compensation for participation in clinical trials (Amgen; Amgen, AbbVie; SK Life Science, AbbVie, Cerevel, respectively). Marcin Straburzyński has also received compensation for scientific consulting (NEUCA Group). Marta Waliszewska-Prosół, PhD, has received honoraria for lectures (Pfizer, AbbVie, Teva). Anna Gryglas-Dworak, PhD, has received compensation for conducting clinical trials (Lundbeck Poland, Amgen, Pfizer, AbbVie, Teva).

**Acknowledgements**
The authors would like to thank: Prof. Richard B. Lipton for his support in the development of the questionnaire and the study protocol; administrators of patient groups on social media sites, the Polish Neurological Society, the Polish Headache Society, the Polish Society of Family Medicine, Premium Clinic Wrocław, NEUCA Group, and Prof. Piotr Dzięgiel, Vice-Chancellor of Wroclaw Medical University for the distribution of the survey; Piotr Prosoł for IT and technical support; Joanna Straburzyńska for English-language editing.

Received on September 30, 2023
Reviewed on November 18, 2023
Accepted on November 21, 2023

Published online on April 23, 2024

**Cite as**
Straburzyński M, Waliszewska-Prosół M, Nowaczewska M, Czapinska-Ciepiela EK, Gryglas-Dworak A, Budrewicz S. Prevalence of cranial autonomic symptoms in frequent episodic tension-type headache: A post hoc analysis of the cross-sectional Migraine in Poland study. *Dent Med Probl.* 2024;61(4):489–493. doi:10.17219/dmp/175611

**DOI**
10.17219/dmp/175611



## Abstract

**Background.** Cranial autonomic symptoms (CASs) include lacrimation, conjunctival injection, rhinorrhea, nasal congestion, facial flushing or sweating, ptosis, and myosis. These symptoms may be associated with trigeminal autonomic cephalalgias (TACs) and migraine.

**Objectives.** The aim of the study was to assess whether CASs are also reported by patients with frequent episodic tension-type headache (eTTH).

**Material and methods.** A cross-sectional online survey of a large Polish population was conducted between August 2021 and June 2022. The analysis assessed diagnostic criteria for migraine and eTTH, as well as the presence of allodynia, headache-related disability and symptoms of depression.

**Results.** The survey involved 3,225 respondents (age: 13–80 years, mean ($M$) = 38.9 years; 87.1% female). A total of 166 individuals met the diagnostic criteria for isolated frequent eTTH without migraine or probable migraine with or without aura. Allodynia was present during the majority of attacks in 40 (24.1%) eTTH subjects, while 86 (51.8%) eTTH respondents reported at least 1 CAS during their headache attacks. The presence of at least 1 CAS was more prevalent in migraine than in eTTH ($p = 0.001$). The respondents with at least 1 CAS during eTTH attacks reported a higher burden associated with pain ($p = 0.024$) and higher Patient Health Questionnaire-9 (PHQ-9) scores ($p = 0.016$).

**Conclusions.** The prevalence of retrospectively reported CASs was high among individuals with eTTH, which may potentially contribute to diagnostic errors. Cranial autonomic symptoms in eTTH do not appear to be caused by severe pain or central sensitization.

**Keywords:** pain, migraine, tears, trigeminal nerve

## Introduction

Headache and facial pain are highly prevalent in the general population.[1] However, these conditions are often accompanied by other symptoms and are rarely limited to pain alone. Trigeminal autonomic cephalalgias (TACs) and migraine may be accompanied by cranial autonomic symptoms (CASs) such as lacrimation, conjunctival injection, rhinorrhea, nasal congestion, facial flushing or sweating, ptosis, and myosis.[2–6] The latter three are considered manifestations of sympathetic pathway activation, while the remaining CASs result from parasympathetic mechanisms.[4,7] There is increasing evidence that parasympathetic CASs may include dysphonia, aural fullness, sneezing, and throat swelling.[6,8] However, these symptoms are not included in the diagnostic criteria for TACs.[9,10] Neurogenic disorders are often associated with symptoms of eye, nose or skin disorders.

At present, the understanding of the mechanisms behind CASs in primary headache disorders points to the superior salivatory nucleus (SSN) as a key region.[7,11] Electrophysiological evidence indicates a direct connection between the trigeminocervical complex (TCC) and SSN in the pons.[6,7] This connection is a fragment of the trigeminal-autonomic reflex (TAR), in which sensory input from trigeminal afferents provokes CASs.[12] By itself, TAR is an important reaction that protects the eyes and upper respiratory tract from harmful situations (e.g., foreign bodies). When triggered by noxious stimuli, the activation spreads from TCC to SSN and further via parasympathetic efferents to the lacrimal glands and nasal mucosa. However, the parasympathetic function is also modulated by input from higher brain structures, including the hypothalamus and limbic and cortical areas,[7,13] which may explain why CASs can occur in primary headache disorders even in the absence of pain (e.g., in the premonitory phase).[14–16] However, CASs are more pronounced when the nociceptive and top-down inputs are combined.[6]

With the exception of trigeminal neuralgia, there is limited evidence to suggest that CASs occur in headache disorders other than TACs and migraine.[17] However, if these symptoms are secondary to TCC activation and subsequent SSN stimulation, then CASs should also be present in tension-type headache (TTH), the most prevalent primary headache disorder. Tension-type headache shares many similarities with migraine, including pain located mainly within the distribution of the first branch of the trigeminal nerve, a paroxysmal and recurring timeline, patient demographics, triggers, and comorbidities.[18] Therefore, CASs in TTH would further support an overlapping etiopathogenesis of these disorders.

The aim of this study was to assess the prevalence of CASs, namely lacrimation, conjunctival injection, ptosis, nasal congestion, rhinorrhea, myosis, and facial flushing or sweating among subjects who meet the diagnostic criteria for frequent episodic TTH (eTTH).

## Material and methods

The Migraine in Poland study is a cross-sectional survey registered in the ClinicalTrials.gov database (registration No. NCT05087420). Data was collected through an online questionnaire distributed via various channels from August 2021 to June 2022, including social media (Facebook, Instagram and Twitter) and national mass media (radio, television, newspapers, websites). The questionnaire was also distributed to employees of Poland's largest state-owned and private companies, state and religious institutions, secondary schools and universities, scientific societies, trade unions and non-governmental organizations, and outpatient service providers in primary and secondary care. The target group was not limited to individuals diagnosed with migraine, although the invitation headline implied a focus on that disorder. The survey assessed diagnostic criteria for migraine and TTH based on the International Classification of Headache Disorders, 3rd edition (ICHD-3). Moreover, the questionnaire examined the presence of CASs listed in the ICHD-3 chapters dedicated to TACs and their relationship to headache attacks. The questionnaire was based on the American Migraine Prevalence and Prevention (AMPP) Study, which enabled the evaluation of respondents' demographic characteristics, headache features and the presence of allodynia.[19] Allodynia was defined as an interictal exacerbation of headache or skin discomfort during normal activities involving sensory stimulation (i.e., combing or pulling hair, face shaving, wearing eyeglasses, contact lenses or earrings, taking a shower, resting face or head on a pillow, and exposure to heat or cold). The study evaluated disease burden using the Migraine Disability Assessment (MIDAS) and assessed the presence and severity of depression using the Patient Health Questionnaire-9 (PHQ-9). The protocol description for the Migraine in Poland study was presented in detail in a previous publication.[20]

### Statistical analysis

Statistical calculations were performed using the R v. 3.6.0 software (https://cran.r-project.org/), PSPP software (https://www.gnu.org/software/pspp/) and Microsoft Office 2019 (Microsoft Corporation, Redmond, USA). The differences between groups were evaluated using a significance level of $p \leq 0.05$. The tests were selected based on the distribution of values, which was verified with the Shapiro–Wilk test. The Pearson's $\chi^2$ test was used to analyze data expressed at the nominal level, with continuity correction applied for 2 × 2 tables. Fisher's exact test was used for tables larger than 2 × 2 when the conditions for the $\chi^2$ test were not met. Quantitative data broken down into groups was analyzed using the Mann–Whitney–Wilcoxon test.

## Results

The survey involved 3,225 respondents (age: 13–80 years, mean (*M*) = 38.9 years; 87.1% female). Of the 1,141 subjects who met the criteria for TTH, 166 respondents met the diagnostic criteria for isolated frequent eTTH according to the ICHD-3 (without co-occurring migraine attacks or probable migraine with or without aura). The ICHD-3 diagnostic criteria for migraine without aura (MwoA) were present in 1,679 participants. Allodynia accompanied the majority of attacks in 40 (24.1%) eTTH subjects.

In the eTTH cohort, 86 (51.8%) respondents reported experiencing at least 1 CAS during their headache attacks. The prevalence of specific CASs and its comparison to the MwoA cohort is presented in Fig. 1. The presence of at least 1 CAS and the presence of at least 2 CASs were significantly more prevalent in respondents with migraine ($p$ = 0.001; $\chi^2$ = 46.656; degrees of freedom (*df*) = 10). Although all CASs were more prevalent in the MwoA group, only conjunctival injection, ptosis and myosis reached statistical significance ($p$ = 0.001).

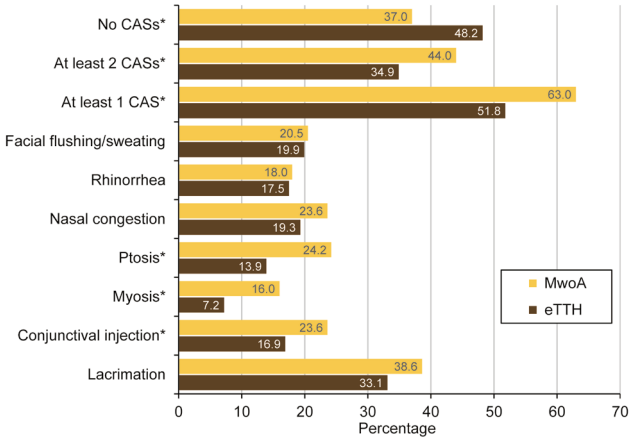


Fig. 1. Interictal cranial autonomic symptoms (CASs) in individuals with episodic tension-type headache (eTTH) and migraine without aura (MwoA)

*statistically significant difference between the eTTH and MwoA groups ($p$ = 0.001; $\chi^2$ = 46.656; degrees of freedom (*df*) = 10).

There were no significant differences in headache intensity expressed on the Numerical Rating Scale between eTTH respondents with and without CASs ($p$ = 0.285; median (*Me*) = 6.0 in both groups; *U* = 3113.0). Headache frequency did not differ between these groups ($p$ = 0.106; *Me* = 14.0 vs. *Me* = 10.0, respectively; *U* = 2939.5). Moreover, allodynia in eTTH was not associated with interictal CASs ($p$ = 0.657; $\chi^2$ = 0.197; *df* = 1). However, patients with at least 1 CAS during an eTTH attack reported a greater burden associated with pain in the MIDAS test ($p$ = 0.024) (Fig. 2). Additionally, subjects with at least 1 CAS had significantly higher PHQ-9 scores ($p$ = 0.016) (Fig. 3).

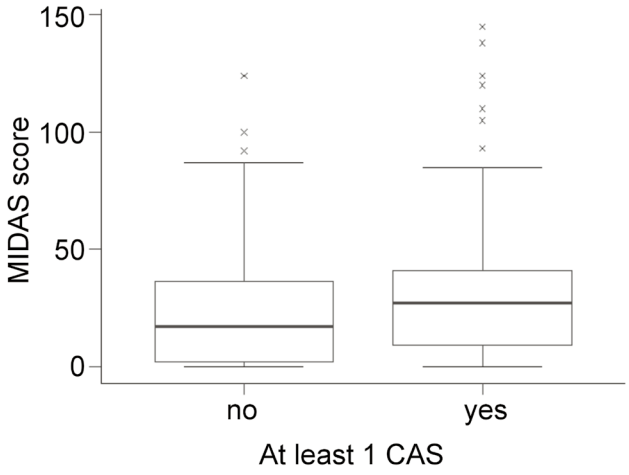


Fig. 2. Distribution of Migraine Disability Assessment (MIDAS) scores in the eTTH group with and without at least 1 CAS ($p$ = 0.024; *U* = 2640.5)

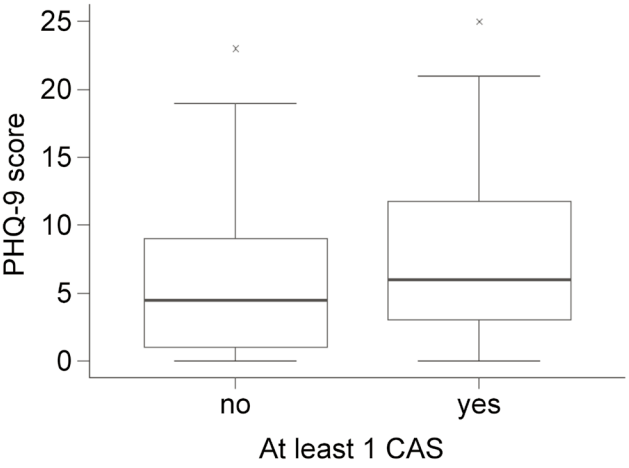


Fig. 3. Distribution of Patient Health Questionnaire (PHQ-9) scores in the eTTH group with and without at least 1 CAS ($p$ = 0.016, *U* = 2695)

## Discussion

Although less common than in MwoA, CASs can also accompany headaches in eTTH. To the best of our knowledge, only 1 study has assessed the prevalence of CASs in TTH.[21] The study compared a selection of CASs (i.e., conjunctival injection, lacrimation, periorbital edema, and nasal symptoms) in 50 subjects with migraine and TTH, and found that only lacrimation and conjunctival injection were reported by TTH subjects. In our study, all canonical CASs were reported interictally by at least some patients with eTTH.

Although our study used convenience sampling, the obtained results are comparable to findings from population-based studies.[20] A recent Danish study reported that 57% and 31% of people with migraine experienced 1 or 2 CASs, respectively.[4] In our study, 1 or 2 CASs interictally were experienced by 63% and 44% of MwoA respondents, respectively. However, in a study by Christensen et al., further cohort validation through telephone interviews

indicated that online questionnaires may overestimate the presence of these symptoms.[4] Future prospective studies using electronic diaries could specify the true prevalence of CASs in migraine or TTH. Nevertheless, the overreporting of CASs is important in clinical practice, as most headache diagnoses rely on retrospective symptom analysis. Therefore, clinicians should be aware that approx. half of patients with TTH or MwoA may report CASs when specifically asked about them. The proposal of the Danish group to require at least 2 interictal CASs may help eliminate overreporting and increase specificity.[4]

Cranial autonomic symptoms in eTTH appear to follow similar patterns as CASs in migraine. For example, Ray et al. found that these symptoms are linked to depression and anxiety in migraine.[22] Similarly, in our study, the presence of CASs was associated with higher PHQ-9 scores. The relationship between depression and pain is well-established in the literature.[23] Cases of CASs in eTTH might be an effect of symptom overreporting by patients with depression or an overlap in etiopathogenesis. Several hypothalamic regions implicated in primary headache disorders with CASs are also involved in mechanisms of depression (e.g., paraventricular[24] and dorsomedial[25] nuclei). Therefore, it is possible that depression contributes to CASs in TTH through hypothalamic involvement and subsequent top-down SSN stimulation.

Some studies indicated that CASs accompany headaches in patients with more severe attacks.[2,5,26–28] If the correlation between pain intensity and CASs is valid, it could explain why these symptoms are more prevalent in migraine than in TTH. Tension-type headache is typically characterized by mild to moderate intensity headaches. Several studies have shown that CASs may also occur in the preictal or postictal phase in cluster headache[14] or migraine,[16] indicating mechanisms other than direct SSN stimulation by TCC.[7] In TTH, where headache intensity is milder, these other mechanisms may come to the forefront since no significant associations were found between headache intensity in TTH and the presence of CAS.

Another important difference between CASs in migraine and TTH is related to central sensitization, which may contribute to chronic TTH mechanisms.[29] Some studies have shown that CASs occur in migraine more often in people with allodynia and other indicators of central sensitization,[13,27] though this observation was mostly valid for patients with very pronounced or multiple CASs.[13] However, allodynia is less prevalent in TTH than in migraine,[30] indicating that central sensitization plays a less important role, at least in episodic cases. In our group, only 24% of patients reported this symptom, and no association with CASs was found.

Questionnaire studies have unavoidable limitations, including recall or cognitive bias. Our survey used convenience sampling, although this was reduced by the broad distribution of the questionnaire. The main study results are similar to population-based surveys, indicating that our sample may be representative. It is important to note that CASs are not specific to headache disorders and may occur in various non-neurological situations (e.g., infections[31]). For example, allergic rhinitis (rhinorrhea and nasal congestion) has a prevalence of 36% in the Polish population,[32] and conjunctivitis (lacrimation and conjunctival injection) occurs in 6–30% of the general European population.[33] These confounding factors may contribute to an overestimation of CAS prevalence. The online nature of the survey may have limited differentiation with less prevalent painful disorders, which could have led to diagnostic inaccuracy, particularly when there is a high overlap of these conditions.[34,35] In an online survey, only the co-occurrence of CAS with headache indicates that what the patient reports is the result of a neurological disorder. Finally, the study was limited by the small number of participants with eTTH, which may be attributed to the fact that the study was advertised as an assessment of migraine. Consequently, patients with eTTH were less likely to participate in the survey.

# Conclusions

The prevalence of retrospectively reported CASs is high in eTTH. Our study results are mostly relevant to the diagnostic side of everyday clinical practice. Cranial autonomic symptoms in patients with TTH may contribute to diagnostic difficulties, as patients and healthcare providers may mistake TTH for other disorders, such as rhinosinusitis (in people with TTH and nasal congestion or rhinorrhea), conjunctivitis (in people with TTH and conjunctival injection or lacrimation) or TAC. Therefore, healthcare providers must be aware that CASs may also be a symptom reported by TTH patients.

The study findings suggest several directions for future research. Firstly, the prevalence of CASs in TTH should be confirmed by prospective diary-based assessment in a clinical setting to exclude recall bias and identify any previously unrevealed concomitant disorders. Secondly, this study highlights neurobiological mechanisms that were not previously assessed in TTH. Therefore, future research should investigate how the activation of TCC may lead to parasympathetic pathway stimulation in TTH.

## Ethics approval and consent to participate

The study was approved by the Bioethics Committee of Wroclaw Medical University, Poland. Participants were required to provide electronic informed consent before starting the questionnaire.

## 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

Marcin Straburzyński https://orcid.org/0000-0002-9811-3526
Marta Waliszewska-Prosół https://orcid.org/0000-0002-9074-2803
Magdalena Nowaczewska https://orcid.org/0000-0002-2304-2903
Ewa Katarzyna Czapinska-Ciepiela https://orcid.org/0009-0008-9493-0263
Anna Gryglas-Dworak https://orcid.org/0000-0002-4430-1804
Slawomir Budrewicz https://orcid.org/0000-0002-2044-6347


### References


1. Steiner TJ, Göbel H, Jensen R, et al. Headache service quality: The role of specialized headache centres within structured headache services, and suggested standards and criteria as centres of excellence. *J Headache Pain*. 2019;20(1):24. doi:10.1186/s10194-019-0970-7
2. Obermann M, Yoon MS, Dommes P, et al. Prevalence of trigeminal autonomic symptoms in migraine: A population-based study. *Cephalalgia*. 2007;27(6):504–509. doi:10.1111/j.1468-2982.2007.01316.x
3. Gelfand AA, Reider AC, Goadsby PJ. Cranial autonomic symptoms in pediatric migraine are the rule, not the exception. *Neurology*. 2013;81(5):431–436. doi:10.1212/WNL.0b013e31829d872a
4. Christensen CG, Techlo TR, Kogelman LJ, et al. Population-based prevalence of cranial autonomic symptoms in migraine and proposed diagnostic appendix criteria. *Cephalalgia*. 2022;42(11–12):1160–1171. doi:10.1177/03331024221094548
5. Barbanti P, Fabbrini G, Pesare M, Vanacore N, Cerbo R. Unilateral cranial autonomic symptoms in migraine. *Cephalalgia*. 2002;22(4):256–259. doi:10.1046/j.1468-2982.2002.00358.x
6. Karsan N, Nagaraj K, Goadsby PJ. Cranial autonomic symptoms: Prevalence, phenotype and laterality in migraine and two potentially new symptoms. *J Headache Pain*. 2022;23(1):18. doi:10.1186/s10194-022-01389-w
7. Akerman S, Goadsby PJ. A novel translational animal model of trigeminal autonomic cephalalgias. *Headache*. 2015;55(1):197–203. doi:10.1111/head.12471
8. Redon S, Donnet A. Sneezing in primary headaches with cranial autonomic symptoms: Pathophysiological considerations. A series of case reports. *Headache*. 2018;58(2):298–303. doi:10.1111/head.13242
9. Headache Classification Committee of the International Headache Society (IHS) The International Classification of Headache Disorders, 3rd edition. *Cephalalgia*. 2018;38(1):1–211. doi:10.1177/0333102417738202
10. International Classification of Orofacial Pain, 1st edition (ICOP). *Cephalalgia*. 2020;40(2):129–221. doi:10.1177/0333102419893823
11. Knight YE, Classey JD, Lasalandra MP, et al. Patterns of fos expression in the rostral medulla and caudal pons evoked by noxious craniovascular stimulation and periaqueductal gray stimulation in the cat. *Brain Res*. 2005;1045(1–2):1–11. doi:10.1016/j.brainres.2005.01.091
12. Frese A, Evers S, May A. Autonomic activation in experimental trigeminal pain. *Cephalalgia*. 2003;23(1):67–68. doi:10.1046/j.1468-2982.2003.00462.x
13. Danno D, Wolf J, Ishizaki K, Kikui S, Hirata K, Takeshima T. Cranial autonomic symptoms in migraine are related to central sensitization: A prospective study of 164 migraine patients at a tertiary headache center. *BMC Neurol*. 2022;22(1):89. doi:10.1186/s12883-022-02610-8
14. Snoer A, Lund N, Beske R, Hagedorn A, Jensen RH, Barloese M. Cluster headache beyond the pain phase: A prospective study of 500 attacks. *Neurology*. 2018;91(9):e822–e831. doi:10.1212/01.wnl.0000542491.92981.03
15. Wei DY, Goadsby PJ. Comprehensive clinical phenotyping of nitroglycerin infusion induced cluster headache attacks. *Cephalalgia*. 2021;41(8):913–933. doi:10.1177/0333102421989617
16. Karsan N, Bose PR, Thompson C, Newman J, Goadsby PJ. Headache and non-headache symptoms provoked by nitroglycerin in migraineurs: A human pharmacological triggering study. *Cephalalgia*. 2020;40(8):828–841. doi:10.1177/0333102420910114
17. Lambru G, Zakrzewska J, Matharu M. Trigeminal neuralgia: A practical guide. *Pract Neurol*. 2021;21(5):392–402. doi:10.1136/practneurol-2020-002782
18. Onan D, Younis S, Wellsgatnik WD, et al. Debate: Differences and similarities between tension-type headache and migraine. *J Headache Pain*. 2023;24(1):92. doi:10.1186/s10194-023-01614-0
19. Silberstein S, Loder E, Diamond S, Reed ML, Bigal ME, Lipton RB; AMPP Advisory Group. Probable migraine in the United States: Results of the American Migraine Prevalence and Prevention (AMPP) study. *Cephalalgia*. 2007;27(3):220–229. doi:10.1111/j.1468-2982.2006.01275.x
20. Waliszewska-Prosół M, Straburzyński M, Czapińska-Ciepiela EK, Nowaczewska M, Gryglas-Dworak A, Budrewicz S. Migraine symptoms, healthcare resources utilization and disease burden in a large Polish migraine cohort: Results from 'Migraine in Poland' – a nationwide cross-sectional survey. *J Headache Pain*. 2023;24(1):40. doi:10.1186/s10194-023-01575-4
21. Gupta R, Bhatia MS. Comparison of clinical characteristics of migraine and tension type headache. *Indian J Psychiatry*. 2011;53(2):134–139. doi:10.4103/0019-5545.82538
22. Ray JC, Cheema S, Foster E, et al. Autonomic symptoms in migraine: Results of a prospective longitudinal study. *Front Neurol*. 2022;13:1036798. doi:10.3389/fneur.2022.1036798
23. Saki M, Shadmanpour M, Najafi HZ. Are individuals with orofacial pain more prone to psychological distress during the COVID-19 pandemic? *Dent Med Probl*. 2021;58(1):17–25. doi:10.17219/dmp/131683
24. Barson JR, Mack NR, Gao WJ. The paraventricular nucleus of the thalamus is an important node in the emotional processing network. *Front Behav Neurosci*. 2020;14:598469. doi:10.3389/fnbeh.2020.598469
25. Nollet M, Gaillard P, Minier F, Tanti A, Belzung C, Leman S. Activation of orexin neurons in dorsomedial/perifornical hypothalamus and antidepressant reversal in a rodent model of depression. *Neuropharmacology*. 2011;61(1–2):336–346. doi:10.1016/j.neuropharm.2011.04.022
26. Lai TH, Fuh JL, Wang SJ. Cranial autonomic symptoms in migraine: Characteristics and comparison with cluster headache. *J Neurol Neurosurg Psychiatry*. 2009;80(10):1116–1119. doi:10.1136/jnnp.2008.157743
27. Barbanti P, Aurilia C, Dall'Armi V, Egeo G, Fofi L, Bonassi S. The phenotype of migraine with unilateral cranial autonomic symptoms documents increased peripheral and central trigeminal sensitization. A case series of 757 patients. *Cephalalgia*. 2016;36(14):1334–1340. doi:10.1177/0333102416630579
28. Togha M, Jafari E, Moosavian A, Farbod A, Ariyanfar S, Farham F. Cranial autonomic symptoms in episodic and chronic migraine: A cross sectional study in Iran. *BMC Neurol*. 2021;21(1):493. doi:10.1186/s12883-021-02513-0
29. Bendtsen L. Central sensitization in tension-type headache-possible pathophysiological mechanisms. *Cephalalgia*. 2000;20(5):486–508. doi:10.1046/j.1468-2982.2000.00070.x
30. Bigal ME, Ashina S, Burstein R, et al. Prevalence and characteristics of allodynia in headache sufferers: A population study. *Neurology*. 2008;70(17):1525–1533. doi:10.1212/01.wnl.0000310645.31020.b1
31. Straburzyński M, Nowaczewska M, Budrewicz S, Waliszewska-Prosół M. COVID-19-related headache and sinonasal inflammation: A longitudinal study analysing the role of acute rhinosinusitis and ICHD-3 classification difficulties in SARS-CoV-2 infection. *Cephalalgia*. 2022;42(3):218–228. doi:10.1177/03331024211040753
32. Samoliński B, Sybilski AJ, Raciborski F, et al. Prevalence of rhinitis in Polish population according to the ECAP (Epidemiology of Allergic Disorders in Poland) study. *Otolaryngol Pol*. 2009;63(4):324–330. doi:10.1016/S0030-6657(09)70135-0
33. Leonardi A, Castegnaro A, Valerio ALG, Lazzarini D. Epidemiology of allergic conjunctivitis: Clinical appearance and treatment patterns in a population-based study. *Curr Opin Allergy Clin Immunol*. 2015;15(5):482–488. doi:10.1097/ACI.0000000000000204
34. Wieckiewicz M, Grychowska N, Nahajowski M, et al. Prevalence and overlaps of headaches and pain-related temporomandibular disorders among the Polish urban population. *J Oral Facial Pain Headache*. 2020;34(1):31–39. doi:10.11607/ofph.2386
35. Seweryn P, Orzeszek SM, Waliszewska-Prosół M, et al. Relationship between pain severity, satisfaction with life and the quality of sleep in Polish adults with temporomandibular disorders. *Dent Med Probl*. 2023;60(4):609–617. doi:10.17219/dmp/171894

Original paper

# Toothbrushing frequency among children and adolescents in 72 countries: Findings from the Global School-based Student Health Survey

Rajat Das Gupta[1,A–F], Rohan Jay Kothadia[2,A,D–F], Shams Shabab Haider[3,E,F], Ananna Mazumder[4,5,E,F], Fatema Akhter[6,E,F], Nazeeba Siddika[5,7,8,E,F], Ehsanul Hoque Apu[5,9,A,D–F]

[1] Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, USA
[2] Arnold School of Public Health, University of South Carolina, Columbia, USA
[3] Friendship NGO, Dhaka, Bangladesh
[4] Jahurul Islam Medical College (JIMC), Kishoreganj, Bangladesh
[5] Centre for International Public Health and Environmental Research, Bangladesh (CIPHER, B), Dhaka, Bangladesh
[6] Department of Surgical and Diagnostic Sciences, College of Dentistry, Dar Al Uloom University, Riyadh, Saudi Arabia
[7] Oral Health Sciences Division, College of Dental Medicine, Lincoln Memorial University, Knoxville, USA
[8] Department of Epidemiology and Biostatistics, College of Human Medicine, Michigan State University, East Lansing, USA
[9] Department of Biomedical Science, College of Dental Medicine, Lincoln Memorial University, Knoxville, 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*. 2024;61(4):495–506

**Address for correspondence**
Rajat Das Gupta
E-mail: rajatdas@email.sc.edu

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on December 14, 2023
Reviewed on February 25, 2024
Accepted on March 8, 2024

Published online on August 9, 2024

**Cite as**
Das Gupta R, Kothadia RJ, Haider SS, et al. Toothbrushing frequency among children and adolescents in 72 countries: Findings from the Global School-based Student Health Survey. *Dent Med Probl.* 2024;61(4):495–506. doi:10.17219/dmp/185842

**DOI**
10.17219/dmp/185842



## Abstract

**Background.** Toothbrushing twice daily is essential for maintaining oral hygiene, which is a cornerstone of overall health. This is particularly important during childhood and adolescence, when lifelong habits are established. Nevertheless, many children and adolescents worldwide face challenges in maintaining good oral health due to limited access to resources and education.

**Objectives.** This study used nationally representative samples from the Global School-based Student Health Survey (GSHS) (2010–2019) to determine the frequency of toothbrushing among school-going students ($N = 266{,}113$) in 72 countries.

**Material and methods.** The country-specific sample size ranged from 130 in Tokelau to 25,408 in Malaysia. The outcome variable was the frequency of brushing or cleaning teeth once daily within the past 30 days prior to the survey. Bivariate analysis was conducted following a descriptive study to determine the frequency of toothbrushing or cleaning across different age groups (≤12, 13, 14, 15, ≥16 years), sexes, World Health Organization (WHO) regions, and gross domestic product (GDP) per capita quintiles.

**Results.** The overall proportion of males to females in the sample was 50.9:49.1. In 45 countries or territories (62.5%), the proportion of participants who reported brushing their teeth at least once a day was above 90%. Participants from 10 countries or territories (13.9%) reported never or rarely brushing their teeth. In 69 countries or territories (95.8%), male students were more likely than female students to never or rarely brush their teeth. The highest rate of individuals who never or rarely brush their teeth (32.1%) was reported in the Eastern Mediterranean Region. In comparison, the Region of the Americas had the highest frequency of brushing twice or more daily (82.9%).

**Conclusions.** Educational interventions focused on dental health implemented in schools and aimed at early adolescents have the potential to promote the formation of healthy habits, which may lead to improved well-being over both short and long terms.

**Keywords:** adolescent, oral hygiene, toothbrushing, global health

# Introduction

Oral hygiene practices are essential for overall health and well-being, especially during childhood and adolescence. Habits formed during these early stages often persist into adulthood.[1] Poor oral health can result in a below-average quality of life, characterized by pain and tooth loss, which many adults experience later in life.[2] Toothbrushing is considered a universal method for preserving oral health and hygiene.[3] The act of brushing twice daily has become a societal standard. Dental professionals and authoritative bodies frequently recommend this regimen.[4] A meta-analysis of 25 studies revealed that individuals who brush their teeth infrequently exhibit a 50% increase in the odds of developing dental caries compared to frequent brushers. Additionally, the analysis demonstrated that individuals who brush their teeth less than twice a day are at a 45% increased risk of developing dental caries compared to those who adhere to a twice-daily brushing regimen.[5] Higher toothbrushing frequency is also associated with a lower risk of periodontal diseases.[6] It is crucial to better understand the global prevalence of toothbrushing frequency, especially among children and adolescents, as this directly impacts oral health, which is an integral component of overall health and well-being. High variability in toothbrushing habits observed across different populations may reflect broader disparities in access to dental care and oral hygiene education.[1] This is particularly important in low- and middle-income countries (LMICs), where the current situation is concerning and data reveals a grim reality. In a previous study, McKittrick and Jacobsen surveyed 146,462 middle school students from 44 LMICs.[1] Of the 44 LMICs included in the study, more than half reported that over 5% of students brushed their teeth less than once a day or not at all. The situation is even more dire in 15 countries, primarily in the Eastern Mediterranean and sub-Saharan Africa regions, where over 10% of students rarely or never engage in dental hygiene practices.[1] The reasons behind the oral health crisis among children and adolescents in these regions are complex. Limited access to oral healthcare services, a lack of knowledge about proper oral hygiene and socioeconomic challenges contribute to the problem.[1] A study in the Dominican Republic, Suriname, and Trinidad and Tobago highlighted substantial deficiencies in oral and hand hygiene among adolescents, which were associated with sociodemographic influences, risky behaviors, mental health issues, and insufficient parental guidance, with few adhering to proper hygiene practices. However, the limited scope of the study to just 3 Caribbean nations calls for a broader investigation into dental hygiene practices across the entire region.[7]

There is a knowledge gap regarding the recent global prevalence of toothbrushing frequency. Therefore, up-to-date data is essential for understanding current trends and challenges in oral health practices. The most recent research reflects the latest societal, economic and environmental changes that could affect oral hygiene habits. The value of this study lies in its potential to guide interventions aimed at improving toothbrushing practices among children and adolescents in LMICs. By understanding the prevalence and causes of inadequate toothbrushing in these settings, policymakers and healthcare professionals can develop targeted strategies to promote good oral hygiene habits. This could significantly improve oral health and the overall well-being of these populations. Focusing on children and adolescents is crucial, as the formation of healthy habits at this stage can impact their long-term well-being. This study aims to assess the frequency of toothbrushing among children and adolescents across 72 countries, utilizing nationally representative samples.

# Material and methods

## Data source

The study employed a nationally representative sample of the Global School-based Student Health Survey (GSHS), conducted in 72 countries from 2010 to 2019. The GSHS is an international collaborative project aimed at facilitating the measurement and evaluation of behavioral risk factors and protective factors in 10 crucial domains among adolescents.[8] The GSHS employs a self-administered questionnaire and is an affordable, school-based survey method that gathers data on the health behaviors and protective factors associated with significant causes of illness and death worldwide, encompassing children and adults. In our study, all GSHS surveys followed a two-stage probability sampling design. At first, the schools were selected based on the probability proportional to size sampling, and then the classrooms were chosen randomly. All students enrolled in a specific class were eligible for inclusion in the study, irrespective of age. They were provided with a self-administered questionnaire. The participants completed the questionnaire in their preferred language under the supervision of competent survey administrators external to the school. In each participating country, the GSHS received ethical approval from either the Ministry of Health or Education, the relevant institutional ethics review committee, or both. Prior to conducting the survey, verbal or written consent was obtained from the participating schools, parents and students in accordance with the established protocols. The study adhered to the ethical principles outlined in the World Medical Association Declaration of Helsinki regarding research involving human subjects.[8]

## Outcome variable

The outcome variable was the frequency of toothbrushing. The participants were asked about the frequency with which they had cleaned or brushed their teeth over the past 30 days. The following 6 options were provided: A. I did not clean or brush my teeth during the past 30 days; B. Less than once per day; C. Once per day; D. 2 times per day; E. 3 times per day; F. 4 or more times per day. The categories A and B were merged to generate a new category representing subjects who either never or rarely brushed or cleaned their teeth. We also merged the categories D, E and F to generate a "twice or more" category.

## Statistical analysis

All analyses were conducted using STATA v. 18.0 (StataCorp LLC, College Station, USA). The survey data from each country was analyzed separately. The survey weight was adjusted throughout the course of the study. At first, descriptive analyses were conducted to determine the frequency of toothbrushing or cleaning among the participants. Then, bivariate analyses were carried out to assess the distribution of brushing or cleaning teeth according to age groups and sexes. The Rao–Scott $\chi^2$ test was conducted to compare the frequency of never or rarely brushing or cleaning teeth across age groups and sexes. The study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines. The gross domestic product (GDP) per capita data from the survey year was retrieved from the World Bank or Index Mundi (when the World Bank data was unavailable), and it was categorized into quintiles (with quintile 1 representing the lowest and quintile 5 representing the highest values).[9,10] We also conducted subgroup analyses for the entire sample by age (≤12, 13, 14, 15, and ≥16 years old), sex (male/female), World Health Organization (WHO) regions (South-East Asian Region, Western Pacific Region, Eastern Mediterranean Region, African Region, Region of the Americas), and GDP per capita (quintile 1–5).

# Results

In total, data from 266,113 participants was included in the study. The country-specific sample size ranged from 130 in Tokelau to 25,408 in Malaysia. In most countries, the number of male participants was higher than that of female participants. The overall proportion of males to females in the sample was 50.9:49.1. The largest proportion of participants were aged ≥16 years (28.6%) (Table 1).

**Table 1.** Background characteristics of the participants

| Region | Country | Year | Sample size, *n* | Sex | | Age [years] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | male [%] | female [%] | ≤12 [%] | 13 [%] | 14 [%] | 15 [%] | ≥16 [%] |
| African Region (*n* = 41,809) | Algeria | 2011 | 4,436 | 48.0 | 52.0 | 20.8 | 18.3 | 18.5 | 23.7 | 18.7 |
| | Benin | 2016 | 2,508 | 73.0 | 27.0 | 1.2 | 4.1 | 7.8 | 11.8 | 75.1 |
| | Eswatini | 2013 | 3,590 | 48.6 | 51.4 | 1.6 | 8.1 | 13.4 | 15.9 | 61.1 |
| | Ghana | 2012 | 3,555 | 51.3 | 48.7 | 7.3 | 9.9 | 14.0 | 15.6 | 53.3 |
| | Liberia | 2017 | 2,529 | 52.0 | 48.0 | 3.8 | 4.1 | 7.6 | 8.9 | 75.7 |
| | Mauritania | 2010 | 1,948 | 54.6 | 45.4 | 5.9 | 9.4 | 19.8 | 29.2 | 35.8 |
| | Mauritius | 2017 | 2,990 | 46.4 | 53.6 | 0.5 | 22.5 | 20.3 | 18.9 | 37.8 |
| | Mozambique | 2015 | 1,811 | 53.3 | 46.7 | 6.0 | 9.5 | 12.4 | 21.1 | 51.1 |
| | Namibia | 2013 | 4,417 | 46.8 | 53.2 | 1.9 | 10.3 | 12.8 | 16.5 | 58.6 |
| | Sierra Leone | 2017 | 2,714 | 51.7 | 48.4 | 3.9 | 13.4 | 17.9 | 19.8 | 45.0 |
| | Sudan | 2012 | 2,079 | 52.9 | 47.2 | 1.1 | 14.9 | 23.6 | 28.5 | 31.8 |
| | United Republic of Tanzania | 2014 | 3,651 | 49.1 | 50.9 | 20.0 | 20.1 | 20.6 | 20.4 | 18.9 |
| | Zimbabwe | 2013 | 5,581 | 50.3 | 49.7 | 1.4 | 12.4 | 25.7 | 31.6 | 28.9 |

| Region | Country | Year | Sample size, *n* | Sex | | Age [years] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | male [%] | female [%] | ≤12 [%] | 13 [%] | 14 [%] | 15 [%] | ≥16 [%] |
| Region of the Americas (n = 48,158) | Bahamas | 2013 | 1,344 | 48.0 | 52.0 | 18.3 | 32.5 | 31.7 | 13.8 | 3.7 |
| | Barbados | 2011 | 1,617 | 49.6 | 50.4 | 2.0 | 20.7 | 36.1 | 33.1 | 8.1 |
| | Belize | 2011 | 2,071 | 48.2 | 51.8 | 22.2 | 19.7 | 21.7 | 19.3 | 17.1 |
| | Bolivia | 2018 | 7,633 | 50.6 | 49.4 | 0.8 | 10.7 | 18.7 | 20.4 | 49.4 |
| | Curaçao | 2015 | 2,685 | 48.8 | 51.2 | 6.7 | 9.5 | 16.4 | 18.1 | 49.3 |
| | Dominican Republic | 2016 | 1,419 | 49.3 | 50.7 | 1.9 | 6.7 | 16.6 | 23.3 | 51.5 |
| | El Salvador | 2013 | 1,847 | 51.5 | 48.6 | 3.8 | 23.1 | 31.7 | 28.2 | 13.2 |
| | Guatemala | 2015 | 4,212 | 52.3 | 47.7 | 6.3 | 21.7 | 28.1 | 26.1 | 17.9 |
| | Honduras | 2012 | 1,736 | 46.8 | 53.2 | 16.2 | 25.7 | 25.9 | 18.9 | 13.3 |
| | Panama | 2018 | 2,874 | 47.3 | 52.7 | 0.4 | 12.1 | 20.8 | 20.0 | 46.7 |
| | Paraguay | 2017 | 3,044 | 48.9 | 51.1 | 5.6 | 15.9 | 21.0 | 19.6 | 37.9 |
| | Peru | 2011 | 2,851 | 50.5 | 49.5 | 1.9 | 19.6 | 30.3 | 32.0 | 16.2 |
| | Saint Kits and Nevis | 2010 | 1,713 | 51.3 | 48.7 | 0.8 | 17.7 | 31.6 | 32.0 | 18.0 |
| | Saint Lucia | 2018 | 1,945 | 47.9 | 52.1 | 17.5 | 18.3 | 18.1 | 17.1 | 29.1 |
| | Saint Vincent and the Grenadines | 2018 | 1,838 | 48.7 | 51.3 | 0.4 | 9.2 | 21.1 | 23.7 | 45.6 |
| | Suriname | 2016 | 2,104 | 49.2 | 50.8 | 7.2 | 17.7 | 23.5 | 20.2 | 31.4 |
| | Trinidad and Tobago | 2017 | 3,801 | 48.0 | 52.0 | 17.5 | 18.5 | 19.2 | 19.1 | 25.7 |
| | Uruguay | 2012 | 3,424 | 45.9 | 54.1 | 0.6 | 22.2 | 30.1 | 30.2 | 16.8 |
| Eastern Mediterranean Region (*n* = 59,956) | Afghanistan | 2014 | 2,356 | 55.7 | 44.3 | 3.4 | 11.8 | 20.4 | 20.3 | 44.2 |
| | Bahrain | 2016 | 7,072 | 50.8 | 49.2 | 13.8 | 20.7 | 20.0 | 19.6 | 25.9 |
| | Morocco | 2016 | 6,477 | 53.9 | 46.2 | 11.5 | 15.2 | 16.6 | 15.2 | 41.4 |
| | Egypt | 2012 | 2,421 | 49.8 | 50.2 | 15.4 | 36.3 | 29.2 | 16.4 | 2.8 |
| | Iraq | 2011 | 1,982 | 56.9 | 43.1 | 7.2 | 21.2 | 22.8 | 24.6 | 24.2 |
| | Kuwait | 2015 | 3,452 | 48.9 | 51.1 | 1.4 | 15.0 | 19.0 | 21.4 | 43.2 |
| | Lebanon | 2017 | 5,662 | 46.9 | 53.1 | 14.1 | 17.1 | 19.1 | 16.0 | 33.7 |
| | Oman | 2015 | 3,389 | 49.8 | 50.2 | 1.7 | 10.3 | 17.3 | 21.1 | 49.6 |
| | Palestine | 2010 | 14,045 | 49.7 | 50.3 | 9.3 | 28.0 | 33.0 | 26.4 | 3.4 |
| | Qatar | 2011 | 1,879 | 48.5 | 51.5 | 24.1 | 29.2 | 29.3 | 13.6 | 3.8 |
| | Syrian Arab Republic | 2011 | 3,061 | 51.2 | 48.8 | 17.4 | 28.7 | 29.8 | 20.7 | 3.4 |
| | United Arab Emirates | 2016 | 5,709 | 49.3 | 50.7 | 5.2 | 14.0 | 19.7 | 20.0 | 41.1 |
| | Yemen | 2014 | 2,451 | 55.1 | 44.9 | 7.9 | 15.9 | 17.7 | 18.3 | 40.2 |
| South-East Asian Region (*n* = 43,102) | Bangladesh | 2014 | 2,972 | 65.2 | 34.8 | 2.5 | 25.1 | 38.0 | 26.0 | 8.4 |
| | Bhutan | 2016 | 7,432 | 48.0 | 52.0 | 4.5 | 10.0 | 14.4 | 16.2 | 54.9 |
| | Indonesia | 2015 | 11,032 | 48.9 | 51.2 | 19.4 | 24.2 | 24.1 | 14.7 | 17.7 |
| | Myanmar | 2016 | 2,796 | 46.6 | 53.4 | 11.6 | 27.0 | 28.8 | 18.3 | 14.3 |
| | Nepal | 2015 | 6,356 | 48.6 | 51.4 | 11.9 | 19.7 | 24.4 | 20.2 | 23.8 |
| | Sri Lanka | 2016 | 3,226 | 48.8 | 51.2 | 2.0 | 21.2 | 24.2 | 21.3 | 31.3 |
| | Thailand | 2015 | 5,836 | 47.0 | 53.0 | 10.1 | 20.4 | 21.2 | 17.2 | 31.1 |
| | Timor-Leste | 2015 | 3,452 | 50.5 | 49.5 | 5.2 | 6.4 | 12.7 | 15.3 | 60.5 |

| Region | Country | Year | Sample size, *n* | Sex | | Age [years] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | male [%] | female [%] | ≤12 [%] | 13 [%] | 14 [%] | 15 [%] | ≥16 [%] |
| Western Pacific Region (*n* = 73,088) | Brunei | 2014 | 2,567 | 50.1 | 49.9 | 3.7 | 18.5 | 26.6 | 22.4 | 28.9 |
| | Cambodia | 2013 | 3,791 | 52.3 | 47.7 | 2.3 | 11.8 | 18.8 | 18.7 | 48.4 |
| | Cook Islands | 2015 | 691 | 48.5 | 51.5 | 0.4 | 9.1 | 22.8 | 20.9 | 46.8 |
| | Fiji | 2016 | 3,558 | 49.1 | 50.9 | 0.6 | 3.6 | 18.5 | 22.2 | 55.1 |
| | French Polynesia | 2015 | 3,188 | 49.6 | 50.4 | 10.7 | 19.2 | 17.0 | 18.0 | 35.2 |
| | Kiribati | 2011 | 1,549 | 47.2 | 52.8 | 3.2 | 21.6 | 31.0 | 31.0 | 13.2 |
| | Laos | 2015 | 3,640 | 53.3 | 46.7 | 0.2 | 4.3 | 11.4 | 22.9 | 61.3 |
| | Malaysia | 2012 | 25,408 | 50.1 | 49.9 | 0.8 | 20.4 | 20.6 | 20.2 | 38.1 |
| | Mongolia | 2013 | 5,343 | 48.3 | 51.8 | 10.7 | 19.7 | 18.8 | 17.8 | 33.0 |
| | Nauru | 2011 | 525 | 46.3 | 53.7 | 14.1 | 21.2 | 22.2 | 17.5 | 25.1 |
| | Niue | 2010 | 140 | 58.2 | 41.8 | 26.6 | 10.0 | 11.7 | 16.2 | 35.6 |
| | Philippines | 2015 | 8,747 | 49.4 | 50.6 | 6.7 | 19.3 | 23.7 | 23.3 | 27.0 |
| | Samoa | 2017 | 1,875 | 47.9 | 52.1 | 9.1 | 14.0 | 18.2 | 17.4 | 41.4 |
| | Solomon Islands | 2011 | 1,303 | 53.9 | 46.1 | 6.4 | 14.7 | 22.8 | 28.0 | 28.1 |
| | Tokelau | 2014 | 130 | 53.6 | 46.4 | 25.6 | 20.4 | 13.1 | 14.8 | 26.1 |
| | Tonga | 2017 | 3,259 | 50.7 | 49.3 | 23.2 | 15.9 | 17.2 | 17.1 | 26.6 |
| | Tuvalu | 2013 | 895 | 48.5 | 51.5 | 21.1 | 20.3 | 17.6 | 13.7 | 27.3 |
| | Vanuatu | 2016 | 2,075 | 49.5 | 50.5 | 3.3 | 11.5 | 18.8 | 22.4 | 44.0 |
| | Vietnam | 2013 | 3,312 | 46.8 | 53.2 | 0.1 | 0.4 | 21.0 | 22.5 | 56.0 |
| | Wallis and Futuna | 2015 | 1,092 | 48.8 | 51.2 | 12.7 | 13.1 | 17.8 | 18.2 | 38.2 |
| Total | | – | 266,113 | 50.9 | 49.1 | 9.4 | 18.7 | 23.3 | 20.0 | 28.6 |

Unweighted frequencies and weighted percentages are reported.

Table 2 illustrates the distribution of participants according to their daily frequency of toothbrushing or cleaning. Overall, 18.8% of participants reported brushing their teeth once a day during the 30 days before the survey, while 70.4% of participants brushed their teeth at least twice a day. The percentage of respondents who reported never cleaning or brushing their teeth ranged from 0.4% in Paraguay to 19.3% in Egypt. In 6 countries, the percentage of respondents who reported never cleaning or brushing their teeth was higher than 10%. These countries were Egypt (19.3%), Yemen (17.8%), Morocco (15.1%), Palestine (13.1%), Syrian Arab Republic (11.2%), and Algeria (10.8%). The proportion of participants who reported cleaning or brushing their teeth at least twice per day ranged from 32.0% in Egypt to 90.1% in Belize. In 16 countries, the percentage of respondents who reported cleaning or brushing their teeth at least twice daily was above 85%. These countries included Belize (90.1%), Paraguay (90.0%), Brunei (89.6%), Indonesia (89.2%), El Salvador (89.1%), Laos (88.6%), Guatemala (88.5%), Uruguay (88.3%), Honduras (87.8%), Barbados (87.3%), Panama (87.0%), Malaysia (86.6%), Tuvalu (85.9%), the Dominican Republic (85.7%), Suriname (85.7%), and the Philippines (85.2%).

Table 3 illustrates the distribution of participants who reported never or rarely brushing or cleaning their teeth, stratified by sex and age. Overall, the percentage ranged from 2.1% in Belize to 39.3% in Egypt. In 56 countries, the prevalence was higher among male participants. In 32 countries, the age group of ≤12 years demonstrated the highest prevalence of never or rarely brushing or cleaning teeth. For the age group of ≥16 years, 13 countries exhibited the highest prevalence of never or rarely brushing or cleaning teeth. There were no clear trends between the age group and the prevalence of never or rarely brushing or cleaning teeth.

The distribution of the percentages of individuals based on the daily frequency of toothbrushing or cleaning, stratified by age, sex, WHO regions, and GDP quintiles is shown in Table 4. The frequency of toothbrushing or cleaning increases with age. The individuals aged

**Table 2.** Distribution of the participants according to the daily frequency of toothbrushing or cleaning

| Country | Daily frequency of toothbrushing or cleaning | | | | | |
|---|---|---|---|---|---|---|
| | Never [%] | <1 [%] | 1 [%] | 2 [%] | 3 [%] | ≥4 [%] |
| Algeria | 10.8 | 9.9 | 20.5 | 24.5 | 21.2 | 13.2 |
| Benin | 0.6 | 3.0 | 24.3 | 35.4 | 26.2 | 10.5 |
| Eswatini | 1.8 | 2.7 | 31.3 | 43.2 | 17.1 | 3.9 |
| Ghana | 2.4 | 5.8 | 28.7 | 43.6 | 9.4 | 10.0 |
| Liberia | 4.5 | 7.6 | 16.9 | 47.1 | 13.9 | 10.1 |
| Mauritania | 4.6 | 10.6 | 21.7 | 17.7 | 21.5 | 24.0 |
| Mauritius | 0.8 | 2.0 | 24.7 | 58.5 | 9.0 | 5.0 |
| Mozambique | 2.3 | 3.9 | 13.8 | 31.1 | 31.3 | 17.7 |
| Namibia | 3.6 | 6.8 | 23.0 | 29.6 | 20.4 | 16.6 |
| Sierra Leone | 2.3 | 5.3 | 21.2 | 42.6 | 21.8 | 6.9 |
| Sudan | 2.8 | 7.2 | 37.3 | 28.7 | 11.7 | 12.4 |
| United Republic of Tanzania | 6.2 | 9.2 | 25.9 | 20.5 | 12.8 | 25.4 |
| Zimbabwe | 3.2 | 3.3 | 12.1 | 26.1 | 55.3 | 0.0 |
| Bahamas | 1.8 | 4.2 | 20.7 | 46.1 | 17.3 | 9.9 |
| Barbados | 1.4 | 2.2 | 9.2 | 57.3 | 23.0 | 7.0 |
| Belize | 0.6 | 1.5 | 7.9 | 36.2 | 40.2 | 13.7 |
| Bolivia | 3.3 | 7.6 | 20.0 | 31.8 | 28.0 | 9.3 |
| Curaçao | 1.4 | 2.4 | 22.3 | 52.0 | 17.3 | 4.5 |
| Dominican Republic | 1.8 | 3.0 | 9.7 | 37.0 | 38.9 | 9.8 |
| El Salvador | 1.0 | 2.0 | 8.0 | 29.4 | 47.4 | 12.3 |
| Guatemala | 2.4 | 3.6 | 5.5 | 14.7 | 55.7 | 18.1 |
| Honduras | 1.7 | 2.4 | 8.1 | 21.4 | 50.2 | 16.2 |
| Panama | 0.5 | 1.8 | 10.6 | 40.3 | 36.1 | 10.6 |
| Paraguay | 0.4 | 2.2 | 7.3 | 23.8 | 49.0 | 17.2 |
| Peru | 1.3 | 3.1 | 13.0 | 29.5 | 43.2 | 9.9 |
| Saint Kits and Nevis | 1.2 | 3.1 | 13.0 | 48.8 | 24.7 | 9.2 |
| Saint Lucia | 1.7 | 3.0 | 12.5 | 54.3 | 20.7 | 7.9 |
| Saint Vincent and the Grenadines | 1.0 | 2.5 | 14.6 | 55.6 | 18.9 | 7.3 |
| Suriname | 1.3 | 1.8 | 11.3 | 48.0 | 28.7 | 9.0 |
| Trinidad and Tobago | 2.3 | 2.7 | 17.0 | 55.6 | 16.2 | 6.1 |
| Uruguay | 0.7 | 1.9 | 9.2 | 28.5 | 39.3 | 20.5 |
| Afghanistan | 9.6 | 18.5 | 33.9 | 16.1 | 10.5 | 11.4 |
| Bahrain | 3.8 | 10.2 | 23.2 | 36.4 | 17.3 | 9.0 |
| Morocco | 15.1 | 17.3 | 21.9 | 18.4 | 15.1 | 12.1 |
| Egypt | 19.3 | 20.0 | 28.7 | 16.8 | 6.5 | 8.7 |
| Iraq | 9.5 | 13.0 | 27.6 | 22.0 | 17.7 | 10.2 |
| Kuwait | 6.4 | 8.8 | 25.7 | 35.2 | 18.4 | 5.5 |
| Lebanon | 3.3 | 5.3 | 26.3 | 37.7 | 21.2 | 6.2 |
| Oman | 4.2 | 9.6 | 30.2 | 34.9 | 13.8 | 7.4 |
| Palestine | 13.1 | 15.3 | 22.9 | 25.7 | 14.1 | 8.9 |
| Qatar | 7.7 | 12.4 | 17.3 | 25.8 | 17.7 | 19.1 |
| Syrian Arab Republic | 11.2 | 22.4 | 26.2 | 22.5 | 13.1 | 4.7 |
| United Arab Emirates | 3.0 | 9.6 | 24.5 | 42.2 | 12.8 | 8.0 |
| Yemen | 17.8 | 18.2 | 26.8 | 12.9 | 12.8 | 11.6 |
| Bangladesh | 1.2 | 11.7 | 23.6 | 52.4 | 10.2 | 0.9 |
| Bhutan | 2.1 | 5.4 | 50.8 | 28.7 | 4.7 | 8.3 |
| Indonesia | 0.5 | 1.8 | 8.5 | 55.4 | 30.5 | 3.3 |
| Myanmar | 1.4 | 4.7 | 33.3 | 45.6 | 12.8 | 2.2 |
| Nepal | 4.8 | 9.9 | 37.0 | 42.9 | 4.3 | 1.2 |
| Sri Lanka | 1.0 | 1.9 | 27.6 | 57.3 | 9.7 | 2.5 |
| Thailand | 2.5 | 2.6 | 11.2 | 66.6 | 13.6 | 3.7 |
| Timor-Leste | 3.8 | 11.1 | 13.3 | 31.1 | 35.7 | 4.9 |
| Brunei | 0.5 | 1.9 | 8.1 | 37.4 | 41.5 | 10.7 |
| Cambodia | 1.0 | 2.9 | 16.2 | 44.5 | 33.9 | 1.4 |
| Cook Islands | 2.4 | 9.6 | 23.3 | 41.5 | 13.6 | 9.6 |
| Fiji | 0.9 | 2.2 | 12.2 | 45.5 | 25.8 | 13.5 |
| French Polynesia | 1.6 | 5.5 | 15.0 | 48.0 | 17.2 | 12.8 |
| Kiribati | 6.6 | 12.2 | 16.3 | 35.9 | 14.9 | 14.1 |
| Laos | 0.5 | 1.8 | 9.2 | 47.7 | 38.0 | 2.9 |
| Malaysia | 0.6 | 2.1 | 10.8 | 41.3 | 34.4 | 10.9 |
| Mongolia | 1.5 | 3.7 | 27.7 | 42.7 | 14.9 | 9.4 |
| Nauru | 4.4 | 8.2 | 18.3 | 25.5 | 13.5 | 30.1 |
| Niue | 3.4 | 6.2 | 24.8 | 42.8 | 12.6 | 10.4 |
| Philippines | 1.5 | 4.0 | 9.3 | 31.1 | 46.2 | 7.9 |
| Samoa | 0.7 | 3.7 | 11.7 | 38.3 | 26.9 | 18.9 |
| Solomon Islands | 7.9 | 15.4 | 18.3 | 21.4 | 18.2 | 18.9 |
| Tokelau | 3.1 | 13.5 | 17.7 | 35.2 | 16.4 | 14.2 |
| Tonga | 2.7 | 5.0 | 12.8 | 30.0 | 8.5 | 41.0 |
| Tuvalu | 3.1 | 3.9 | 7.2 | 18.7 | 35.0 | 32.2 |
| Vanuatu | 3.3 | 7.1 | 16.9 | 20.8 | 33.9 | 18.1 |
| Vietnam | 1.1 | 1.6 | 20.5 | 64.3 | 11.4 | 1.1 |
| Wallis and Futuna | 2.4 | 6.3 | 11.7 | 34.7 | 29.7 | 15.2 |

**Table 3.** Distribution of the participants who reported never or rarely brushing or cleaning their teeth, stratified by sex and age

| Country | Total [%] | Sex | | | Age [years] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | male [%] | female [%] | *p*-value | ≤12 [%] | 13 [%] | 14 [%] | 15 [%] | ≥16 [%] | *p*-value |
| Algeria | 20.6 | 24.9 | 16.7 | <0.001* | 17.0 | 19.6 | 21.3 | 20.4 | 25.3 | 0.031* |
| Benin | 3.6 | 4.2 | 2.1 | <0.01* | 1.3 | 4.3 | 1.7 | 3.5 | 3.8 | 0.381 |
| Eswatini | 4.5 | 5.9 | 3.1 | <0.01* | 1.7 | 6.0 | 5.4 | 4.3 | 4.2 | 0.388 |
| Ghana | 8.2 | 8.1 | 8.4 | 0.752 | 15.7 | 9.5 | 10.2 | 11.4 | 5.5 | 0.008* |
| Liberia | 12.1 | 13.1 | 11.0 | 0.140 | 27.9 | 17.2 | 10.0 | 15.2 | 10.9 | <0.001* |
| Mauritania | 15.3 | 17.0 | 13.1 | <0.01* | 16.5 | 10.7 | 14.7 | 15.7 | 16.2 | 0.393 |
| Mauritius | 2.8 | 3.4 | 2.2 | 0.223 | 0.0 | 3.4 | 2.1 | 2.7 | 2.8 | 0.790 |
| Mozambique | 6.2 | 6.3 | 6.0 | 0.784 | 10.2 | 9.5 | 0.7 | 7.4 | 5.9 | 0.033* |
| Namibia | 10.4 | 11.1 | 9.8 | 0.197 | 16.5 | 13.9 | 12.1 | 11.7 | 8.9 | 0.030* |
| Sierra Leone | 7.6 | 8.6 | 6.5 | 0.106 | 7.0 | 6.1 | 9.1 | 8.2 | 7.2 | 0.689 |
| Sudan | 10.0 | 12.1 | 7.6 | 0.105 | 1.8 | 7.2 | 11.3 | 11.1 | 9.4 | 0.478 |
| United Republic of Tanzania | 15.4 | 16.0 | 14.8 | 0.323 | 25.1 | 14.6 | 15.0 | 10.3 | 11.9 | <0.001* |
| Zimbabwe | 6.5 | 8.2 | 4.8 | <0.001* | 9.2 | 5.8 | 5.7 | 6.7 | 7.3 | 0.437 |
| Bahamas | 6.0 | 8.2 | 3.9 | <0.001* | 6.0 | 6.3 | 5.3 | 6.4 | 7.7 | 0.893 |
| Barbados | 3.5 | 4.2 | 2.9 | 0.251 | 7.1 | 3.2 | 4.5 | 2.9 | 2.3 | 0.448 |
| Belize | 2.1 | 2.6 | 1.7 | 0.115 | 1.3 | 2.0 | 2.4 | 1.9 | 3.0 | 0.560 |
| Bolivia | 10.9 | 13.2 | 8.6 | <0.001* | 23.7 | 11.6 | 11.1 | 10.6 | 10.6 | 0.059 |
| Curaçao | 3.8 | 4.6 | 3.1 | 0.060 | 4.0 | 3.8 | 4.1 | 3.4 | 3.9 | 0.966 |
| Dominican Republic | 4.7 | 6.4 | 3.1 | 0.013* | 34.4 | 3.1 | 5.9 | 5.4 | 3.1 | 0.001* |
| El Salvador | 2.9 | 3.5 | 2.3 | 0.060 | 5.2 | 3.2 | 1.8 | 2.7 | 5.1 | 0.091 |
| Guatemala | 6.0 | 6.3 | 5.7 | 0.712 | 7.9 | 3.1 | 6.8 | 6.5 | 6.8 | 0.147 |
| Honduras | 4.1 | 5.0 | 3.2 | 0.067 | 4.9 | 4.9 | 2.9 | 3.4 | 4.8 | 0.379 |
| Panama | 2.3 | 2.4 | 2.3 | 0.835 | 9.3 | 2.0 | 3.2 | 1.9 | 2.2 | 0.288 |
| Paraguay | 2.6 | 3.4 | 1.9 | 0.028* | 3.7 | 2.7 | 1.9 | 1.8 | 3.3 | 0.164 |
| Peru | 4.4 | 5.1 | 3.8 | 0.290 | 1.8 | 3.3 | 5.0 | 4.3 | 5.2 | 0.449 |
| Saint Kits and Nevis | 4.3 | 5.4 | 3.1 | <0.001* | 5.5 | 3.6 | 5.0 | 3.1 | 5.7 | <0.001* |
| Saint Lucia | 4.7 | 6.7 | 2.9 | <0.002* | 4.7 | 5.8 | 3.9 | 6.0 | 3.7 | 0.626 |
| Saint Vincent and the Grenadines | 3.5 | 4.2 | 2.9 | 0.131 | 11.8 | 2.7 | 3.0 | 2.1 | 4.6 | 0.076 |
| Suriname | 3.1 | 3.6 | 2.5 | 0.160 | 0.0 | 1.7 | 2.4 | 4.2 | 4.3 | 0.104 |
| Trinidad and Tobago | 5.0 | 6.6 | 3.6 | 0.016* | 4.3 | 5.5 | 4.1 | 4.9 | 6.0 | 0.662 |
| Uruguay | 2.6 | 3.8 | 1.5 | 0.001* | 8.4 | 2.7 | 2.4 | 2.6 | 2.5 | 0.575 |
| Afghanistan | 28.1 | 35.6 | 18.6 | 0.001* | 19.0 | 23.7 | 23.4 | 29.6 | 31.4 | 0.177 |
| Bahrain | 14.0 | 21.0 | 6.8 | <0.001* | 11.2 | 14.4 | 12.9 | 15.4 | 15.0 | 0.410 |
| Morocco | 32.4 | 38.7 | 25.1 | <0.001* | 25.3 | 37.1 | 32.8 | 34.3 | 31.8 | 0.018* |
| Egypt | 39.3 | 42.1 | 36.6 | 0.282 | 45.9 | 38.4 | 36.1 | 38.3 | 55.3 | 0.509 |
| Iraq | 22.4 | 27.2 | 16.1 | <0.001* | 20.1 | 20.2 | 20.3 | 24.1 | 25.4 | 0.445 |

| Country | Total [%] | Sex | | | Age [years] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | male [%] | female [%] | *p*-value | ≤12 [%] | 13 [%] | 14 [%] | 15 [%] | ≥16 [%] | *p*-value |
| Kuwait | 15.2 | 21.8 | 8.9 | <0.001* | 23.6 | 14.1 | 14.7 | 14.9 | 15.6 | 0.691 |
| Lebanon | 8.6 | 11.7 | 5.8 | <0.001* | 7.3 | 6.1 | 9.0 | 9.3 | 9.7 | 0.085 |
| Oman | 13.8 | 19.8 | 7.8 | <0.001* | 17.5 | 11.8 | 15.1 | 17.1 | 12.3 | 0.157 |
| Palestine | 28.4 | 35.2 | 21.7 | <0.001* | 26.6 | 27.4 | 26.7 | 31.5 | 34.0 | 0.001* |
| Qatar | 20.1 | 27.8 | 12.9 | <0.001* | 20.8 | 15.3 | 20.9 | 28.2 | 16.8 | 0.138 |
| Syrian Arab Republic | 33.6 | 38.2 | 28.9 | <0.001* | 34.5 | 33.5 | 35.8 | 30.3 | 31.9 | 0.515 |
| United Arab Emirates | 12.6 | 19.6 | 5.8 | <0.001* | 9.1 | 9.4 | 12.1 | 13.3 | 14.1 | 0.018* |
| Yemen | 36.0 | 43.6 | 26.6 | <0.001* | 26.3 | 37.1 | 36.5 | 35.6 | 37.3 | 0.168 |
| Bangladesh | 12.9 | 15.2 | 8.5 | 0.002* | 8.3 | 10.9 | 14.3 | 11.0 | 19.1 | 0.124 |
| Bhutan | 7.5 | 8.6 | 6.5 | <0.001* | 7.4 | 8.7 | 7.8 | 7.1 | 7.3 | 0.733 |
| Indonesia | 2.3 | 3.5 | 1.1 | <0.001* | 3.1 | 2.0 | 2.8 | 1.9 | 1.3 | 0.040* |
| Myanmar | 6.1 | 7.5 | 4.9 | 0.019* | 4.8 | 6.7 | 7.1 | 4.2 | 6.6 | 0.226 |
| Nepal | 14.7 | 13.5 | 15.8 | 0.282 | 23.8 | 14.9 | 13.4 | 11.0 | 14.4 | 0.008* |
| Sri Lanka | 3.0 | 3.5 | 2.5 | 0.113 | 2.9 | 4.2 | 2.8 | 2.5 | 2.5 | 0.444 |
| Thailand | 5.0 | 8.3 | 2.1 | <0.001* | 5.1 | 6.2 | 6.1 | 4.0 | 4.0 | 0.183 |
| Timor-Leste | 14.9 | 16.1 | 13.7 | 0.083 | 28.7 | 18.6 | 15.5 | 17.9 | 12.5 | <0.001* |
| Brunei | 2.3 | 2.9 | 1.7 | 0.047* | 4.5 | 2.2 | 2.0 | 2.1 | 2.5 | 0.693 |
| Cambodia | 3.9 | 4.6 | 3.2 | 0.034* | 1.5 | 7.0 | 5.0 | 3.4 | 3.1 | 0.012* |
| Cook Islands | 12.0 | 14.5 | 9.6 | 0.035* | 0.0 | 6.2 | 13.2 | 14.5 | 11.5 | 0.497 |
| Fiji | 3.1 | 4.2 | 2.0 | 0.001* | 10.1 | 4.9 | 3.1 | 3.2 | 2.8 | 0.443 |
| French Polynesia | 7.1 | 9.2 | 4.9 | <0.001* | 7.4 | 9.9 | 6.3 | 8.0 | 5.3 | 0.074 |
| Kiribati | 18.8 | 23.0 | 15.0 | <0.001* | 20.3 | 22.2 | 20.7 | 16.4 | 14.0 | 0.119 |
| Laos | 2.3 | 2.1 | 2.5 | 0.423 | 0.0 | 3.0 | 2.4 | 2.1 | 2.3 | 0.935 |
| Malaysia | 2.7 | 3.7 | 1.7 | <0.001* | 6.6 | 3.3 | 2.6 | 2.6 | 2.4 | 0.052 |
| Mongolia | 5.2 | 6.9 | 3.7 | <0.001* | 6.3 | 6.2 | 5.7 | 4.5 | 4.4 | 0.199 |
| Nauru | 12.6 | 16.5 | 9.2 | <0.001* | 12.4 | 14.7 | 11.4 | 16.2 | 9.4 | <0.001* |
| Niue | 9.5 | 12.0 | 6.1 | <0.001* | 18.4 | 7.9 | 16.0 | 4.6 | 3.5 | <0.001* |
| Philippines | 5.5 | 6.3 | 4.7 | 0.138 | 4.3 | 5.0 | 5.9 | 6.6 | 4.9 | 0.771 |
| Samoa | 4.3 | 5.0 | 3.7 | 0.194 | 8.9 | 4.3 | 4.1 | 5.0 | 3.2 | 0.235 |
| Solomon Islands | 23.3 | 26.9 | 19.0 | 0.003* | 17.2 | 26.9 | 24.7 | 21.9 | 23.1 | 0.640 |
| Tokelau | 16.6 | 20.5 | 12.0 | 0.042* | 24.2 | 13.2 | 16.3 | 13.5 | 13.7 | 0.415 |
| Tonga | 7.7 | 11.8 | 3.4 | <0.001* | 11.0 | 7.0 | 6.5 | 7.9 | 5.8 | 0.021* |
| Tuvalu | 7.0 | 10.9 | 3.3 | <0.001* | 12.2 | 6.1 | 7.8 | 5.4 | 3.9 | <0.001* |
| Vanuatu | 10.4 | 13.3 | 7.6 | <0.001* | 17.8 | 12.2 | 7.4 | 9.1 | 11.3 | 0.142 |
| Vietnam | 2.7 | 3.7 | 1.9 | 0.004* | 0.0 | 6.7 | 4.1 | 2.7 | 2.2 | 0.121 |
| Wallis and Futuna | 8.7 | 11.4 | 6.1 | 0.006* | 12.8 | 9.7 | 7.6 | 9.2 | 7.2 | 0.383 |

* statistically significant ($p < 0.05$, Rao–Scott $\chi^2$ test). The percentage values presented in the table are based on the entire study sample.

**Table 4.** Distribution of the participants based on the daily frequency of toothbrushing or cleaning, stratified by covariates

| Covariates | | Daily frequency of toothbrushing or cleaning | | | *p*-value |
|---|---|---|---|---|---|
| | | never or rarely [%] | once a day [%] | twice or more [%] | |
| Age [years] | ≤12 | 14.2 | 16.4 | 69.3 | <0.001* |
| | 13 | 12.6 | 18.3 | 69.1 | |
| | 14 | 11.5 | 18.4 | 70.2 | |
| | 15 | 10.3 | 19.7 | 69.9 | |
| | ≥16 | 8.6 | 19.5 | 72.0 | |
| Sex | male | 13.1 | 21.3 | 65.7 | <0.001* |
| | female | 8.6 | 16.2 | 75.2 | |
| WHO regions | South-East Asian Region | 6.0 | 16.9 | 77.1 | <0.001* |
| | Western Pacific Region | 3.9 | 14.7 | 81.4 | |
| | Eastern Mediterranean Region | 32.1 | 26.5 | 41.4 | |
| | African Region | 12.3 | 24.7 | 63.0 | |
| | Region of the Americas | 5.4 | 11.6 | 82.9 | |
| GDP per capita | quintile 1 | 13.4 | 26.7 | 59.8 | <0.001* |
| | quintile 2 | 10.5 | 15.2 | 74.3 | |
| | quintile 3 | 10.7 | 20.1 | 69.3 | |
| | quintile 4 | 7.2 | 14.2 | 78.6 | |
| | quintile 5 | 9.3 | 20.8 | 69.9 | |

* statistically significant ($p < 0.05$, Rao–Scott $\chi^2$ test); WHO – World Health Organization; GDP – gross domestic product.

12 years or younger reported the highest rates of never or rarely brushing their teeth (14.2%), which decreased progressively to 8.6% in individuals aged 16 years or older ($p < 0.001$). On the other hand, the proportion of individuals who brushed their teeth twice or more per day increased from 69.3% in the youngest age group to 72.0% in the oldest. Notable sex disparities were observed in the toothbrushing practices. Males reported higher rates of never or rarely brushing their teeth (13.1%) compared to females (8.6%; $p < 0.001$). Conversely, a higher percentage of females (75.2%) reported brushing their teeth twice or more daily compared to males (65.7%; $p < 0.001$). The prevalence of toothbrushing frequency varied significantly across WHO regions ($p < 0.001$). The highest rate of individuals who never or rarely brushed their teeth (32.1%) was observed in the Eastern Mediterranean Region. In comparison, the Region of the Americas had the highest frequency of brushing twice or more daily (82.9%). The lowest GDP quintile (quintile 1) exhibited the highest percentage of individuals who never or rarely brushed their teeth (13.4%), while the frequency of brushing twice or more daily increased with the economic status, reaching a peak in quintile 4 (78.6%). However, quintile 5 showed a slight reduction in the frequency of brushing twice or more daily (69.9%).

## Discussion

This study aimed to determine the prevalence of oral hygiene practices among school-going students in 72 countries across 5 WHO regions. Using nationally representative samples, this study determined that the proportion of individuals who never cleaned their teeth ranged from 0.4% in Paraguay to 19.3% in Egypt. In 6 countries, the prevalence of respondents who never cleaned their teeth exceeded 10%, including Egypt (19.3%), Morocco (15.1%) and Yemen (17.8%). On the other hand, 16 countries had the prevalence of more than 85% of respondents who reported cleaning their teeth at least twice daily, with Belize (90.1%) showing the highest proportions. The prevalence of never or rarely brushing teeth was higher among male participants in 56 countries, while the age group of ≤12 years exhibited the highest prevalence in 32 countries. The frequency of toothbrushing twice daily tends to increase with age and is more common among females, with notable geographical variations. The highest adherence was observed in the Region of the Americas, while the lowest adherence was observed in the Eastern Mediterranean Region. The economic status also influenced brushing habits, with a peak in the higher GDP quintiles. However, the highest quintile shows a slight decrease in frequent brushing.

Approximately 90% of participants brushed their teeth at least once daily, while about 71% of respondents brushed their teeth at least twice daily. These findings are similar to those of a previous study that examined oral hygiene practices in 44 LMICs using the GSHS 2003–2010 data.[1] However, the previous study reported a higher prevalence of participants who brushed their teeth at least once per day, with a reported rate of 98.9%. This discrepancy in the percentages may be due to differences in time and place. The finding is similar to the 2017/2018 Health Behaviour in School-aged Children (HBSC) Survey in Europe and Canada, wherein 65% of adolescents reported brushing their teeth twice a day.[11]

In 56 countries, males were more likely to never or rarely brush their teeth. This aligns with the 2017/2018 HSBC survey, which revealed that European countries demonstrated a higher prevalence of toothbrushing more than once a day among female adolescents compared to their male counterparts.[11] Previous rounds of GSHS surveys (2003–2010) also reported a higher prevalence of healthy oral hygiene among females.[1] Poor oral hygiene practice among boys persists into adulthood. As a result, adult males are more prone to developing periodontal disease than adult females.[12,13] These findings highlight the significance of implementing health education programs that foster healthy dental hygiene behaviors among junior secondary school students, with a particular focus on boys in this age group.

Among the 7 countries with a prevalence of never cleaning or brushing teeth exceeding 5%, 5 were in the Middle East. The GSHS results indicate a notably high rate of rare or never brushing reported in the Middle East and some African countries, which may be attributed to the use of a traditional tooth cleaning stick called miswak.[1] As miswak may not be perceived as a "brushing or cleaning" device, this could have led to an underestimated frequency of reported oral hygiene activities.[1,14,15] Additionally, since Middle Eastern countries had a higher GDP per capita, this could explain why we observed a lower prevalence of toothbrushing twice daily in the quintile 5 compared to quintile 4.

As 29% of participants do not adhere to the recommended twice-daily toothbrushing regimen, they risk developing dental caries and periodontal diseases.[16,17] Health promotion programs should raise awareness among school-going individuals about the health benefits of regular toothbrushing.

Dental caries is a major public health problem among children and adolescents, which negatively impacts their quality of life.[18–20] This condition is associated with impaired cognitive growth, higher rates of school absences, poorer academic achievement, more workdays lost for parents, and a diminished quality of life.[21–23] Promoting healthy behaviors, including regular toothbrushing in school-age children, is important for establishing lifelong habits that contribute to overall well-being.[24] When children learn the importance of maintaining oral hygiene, they are more likely to continue these practices into adulthood, significantly reducing the risk of dental issues and fostering a sense of personal health responsibility. Some studies also suggest that the development of healthy habits should commence at an earlier age.[25,26] Encouraging such habits through educational programs and parental involvement can ensure the sustainability and a healthier future for next generations.[27–29]

The implementation of a policy related to toothbrushing frequency in schools will require a comprehensive step-by-step approach and thorough consideration. This may involve the presentation of statistical data or research findings on the knowledge of oral health among parents and dental hygiene conditions among students, with a particular focus on tooth decay and cavities, linking these metrics to overall health, school attendance, etc.[30,31]

## Limitations

The robustness of the study lies in its consistent methodology, which was applied to nationally representative samples from 72 countries. However, it is important to consider the limitations of the study. Causal inference cannot be applied due to the cross-sectional nature of the survey. The GSHS assessed oral hygiene behavior and relied on self-reporting, which may have introduced reporting bias. Nevertheless, it is worth mentioning that self-reported toothbrushing frequency has been utilized as a proxy indicator for clinical oral hygiene incidences among adolescents in other studies.[32] Finally, there might be a misinterpretation regarding the act of cleaning or brushing teeth among the participants in the Middle East. Future GSHS studies should also include miswak in order to adapt the study to the local context.

Broader research areas, such as other schools, districts and even different countries, should be explored. Information may be obtained from a variety of sources, including scientific research, dental health organizations, the WHO guidelines, etc. Workshops and classes should be conducted by the local dental institutions and dental health professionals on the recommended frequency of toothbrushing, proper brushing techniques, use of appropriate kinds of toothpaste, expected duration of brushing, and proper storage of toothbrushes. Periodic reviews should be completed to assess effectiveness, address potential issues and make necessary revisions. A general guideline may need to be tailored to fit the specific regulations and conditions of each school or district. Further studies are warranted to identify the determinants of oral hygiene practices among school-going students globally. In addition, appropriate collaboration among dental research teams from these countries is needed to form a task force and action plan in order to improve oral health awareness and conditions among schoolchildren.[33]

## Conclusions

The results of the study suggest that 9 out of 10 students brush their teeth at least once a day. However, nearly 30% of the students do not brush their teeth at least twice a day. Females exhibited a higher frequency of brushing their teeth than males. School-based educational programs targeting dental health, especially for early adolescents, can significantly foster the development of positive habits that benefit immediate and future well-being. Further research is required to explore the factors influencing oral hygiene behaviors among students worldwide.

## Ethics approval and consent to participate

In each participating country, the GSHS received ethical approval from either the Ministry of Health or Education, the relevant institutional ethics review committee, or both. Participants were approached for data collection after written or verbal consent was obtained from the adolescents and their respective parents or guardians. Given that our research employed publicly accessible retrospective data, ethical approval was not required.

## Data availability

Data can be accessed from the WHO NCD microdata repository at the following URL: https://extranet.who.int/ncdsmicrodata/index.php/home.

## Consent for publication

Not applicable.

### ORCID iDs

Rajat Das Gupta https://orcid.org/0000-0002-7680-676X
Rohan Jay Kothadia https://orcid.org/0009-0007-1228-9074
Shams Shabab Haider https://orcid.org/0000-0003-4895-253X
Ananna Mazumder https://orcid.org/0009-0001-9028-4603
Fatema Akhter https://orcid.org/0000-0003-4014-2399
Nazeeba Siddika https://orcid.org/0000-0002-5400-1784
Ehsanul Hoque Apu https://orcid.org/0000-0001-6840-3787

### References

1. McKittrick TR, Jacobsen KH. Oral hygiene practices among middle-school students in 44 low- and middle-income countries. *Int Dent J*. 2014;64(3):164–170. doi:10.1111/idj.12094
2. Weintraub JA. The Oral Health in America Report: A public health research perspective. *Prev Chronic Dis*. 2022;19:E58. doi:10.5888/PCD19.220067
3. Poklepovic T, Worthington HV, Johnson TM, et al. Interdental brushing for the prevention and control of periodontal diseases and dental caries in adults. *Cochrane Database Syst Rev*. 2013;2013(12):CD009857. doi:10.1002/14651858.CD009857.PUB2
4. Oral Health | CDC. https://www.cdc.gov/oral-health/index.html. Accessed February 11, 2024.
5. Kumar S, Tadakamadla J, Johnson NW. Effect of toothbrushing frequency on incidence and increment of dental caries: A systematic review and meta-analysis. *J Dent Res*. 2016;95(11):1230–1236. doi:10.1177/0022034516655315
6. Abe M, Mitani A, Zong L, Zhang CD, Hoshi K, Yanagimoto S. High frequency and long duration of toothbrushing can potentially reduce the risk of common systemic diseases in late adolescence. *Spec Care Dentist*. 2022;42(3):317–318. doi:10.1111/SCD.12670
7. Pengpid S, Peltzer K. Hand and oral hygiene practices among adolescents in Dominican Republic, Suriname and Trinidad and Tobago: Prevalence, health, risk behavior, mental health and protective factors. *Int J Environ Res Public Health*. 2020;17(21):7860. doi:10.3390/IJERPH17217860
8. Centers for Disease Control and Prevention (CDC) (2018). GSHS data user's guide. https://archive.cdc.gov/www_cdc_gov/gshs/index.htm. Accessed June 10, 2023.
9. Index Mundi. GDP per capita (current US$). https://www.indexmundi.com/facts/indicators/NY.GDP.PCAP.CD. Accessed February 12, 2023.
10. World Bank Group. GDP per capita (current US$). https://data.worldbank.org/indicator/NY.GDP.PCAP.CD. Accessed February 12, 2023.
11. Inchley J, Currie D, Budisavljevic S, et al., eds. *Spotlight on Adolescent Health and Well-Being. Findings from the 2017/2018 Health Behaviour in School-aged Children (HBSC) Survey in Europe and Canada. International Report. Volume 1. Key Findings*. Copenhagen, Denmark: WHO Regional Office for Europe; 2020:1–55.
12. Lipsky MS, Su S, Crespo CJ, Hung M. Men and oral health: A review of sex and gender differences. *Am J Mens Health*. 2021;15(3):15579883211016361. doi:10.1177/15579883211016361
13. Su S, Lipsky MS, Licari FW, Hung M. Comparing oral health behaviours of men and women in the United States. *J Dent*. 2022;122:104157. doi:10.1016/J.JDENT.2022.104157
14. Aboul-Enein BH. The miswak (*Salvadora persica L.*) chewing stick: Cultural implications in oral health promotion. *Saudi J Dent Res*. 2014;5(1):9–13. doi:10.1016/j.ksujds.2013.06.002
15. Riggs E, van Gemert C, Gussy M, Waters E, Kilpatrick N. Reflections on cultural diversity in oral health promotion and prevention. *Glob Health Promot*. 2012;19(1):60–63. doi:10.1177/1757975911429872
16. Attin T, Hornecker E. Tooth brushing and oral health: How frequently and when should tooth brushing be performed? *Oral Health Prev Dent*. 2005;3(3):135–140. PMID:16355646.
17. Lertpimonchai A, Rattanasiri S, Arj-Ong Vallibhakara S, Attia J, Thakkinstian A. The association between oral hygiene and periodontitis: A systematic review and meta-analysis. *Int Dent J*. 2017;67(6):332–343. doi:10.1111/IDJ.12317
18. Yavsan ZS, Tosun G, Sert A. Oral health-related quality of life of preschool-aged Turkish children with congenital heart disease. *Dent Med Probl*. 2022;59(4):503–508. doi:10.17219/DMP/143581
19. Fernandes SC, Louceiro A, Lopes LB, Esteves F, Arriaga P. Children's attitudes and behaviors about oral health and dental practices. *Healthcare (Basel)*. 2021;9(4):416. doi:10.3390/HEALTHCARE9040416
20. Oral Health Across the Lifespan: Adolescents. In: National Institutes of Health. *Oral Health in America: Advances and Challenges [Internet]*. Bethesda, MD: National Institutes of Health; 2021. https://www.ncbi.nlm.nih.gov/books/NBK578291
21. Sheiham A. Dental caries affects body weight, growth and quality of life in pre-school children. *Br Dent J*. 2006;201(10):625–626. doi:10.1038/SJ.BDJ.4814259
22. Casamassimo PS, Thikkurissy S, Edelstein BL, Maiorini E. Beyond the dmft: The human and economic cost of early childhood caries. *J Am Dent Assoc*. 2009;140(6):650–657. doi:10.14219/JADA.ARCHIVE.2009.0250
23. Guarnizo-Herreño CC, Lyu W, Wehby GL. Children's oral health and academic performance: Evidence of a persisting relationship over the last decade in the United States. *J Pediatr*. 2019;209:183–189.e2. doi:10.1016/J.JPEDS.2019.01.045
24. Şenol Y, Şenol FB. Health promotion in preschool children. *Children (Basel)*. 2023;10(8):1385. doi:10.3390/CHILDREN10081385
25. Janiani P, Ravindran V. Comparative evaluation of the antimicrobial effects of probiotic milk and probiotic powder on the salivary *Streptococcus mutans* counts and the plaque scores in children aged 3–6 years: A randomized controlled trial. *Dent Med Probl*. 2022;59(1):99–104. doi:10.17219/DMP/139731

26. Góngora-León I, Alarcón-Calle CS, Aliaga-Del Castillo A, Flores-Mir C, Arriola-Guillén LE. Association of breastfeeding duration with the development of non-nutritive habits, and transversal and vertical occlusal alterations in preschool children: A cross-sectional study. *Dent Med Probl*. 2023;60(1):47–53. doi:10.17219/DMP/145416
27. Varkey IM, Ghule KD, Mathew R, et al. Assessment of attitudes and practices regarding oral healthcare during the COVID-19 pandemic among the parents of children aged 4–7 years. *Dent Med Probl*. 2022;59(3):365–372. doi:10.17219/DMP/147184
28. Duijster D, de Jong-Lenters M, Verrips E, van Loveren C. Establishing oral health promoting behaviours in children – parents' views on barriers, facilitators and professional support: A qualitative study. *BMC Oral Health*. 2015;15:157. doi:10.1186/S12903-015-0145-0
29. Sowmiya Sree RA, Joe Louis C, Senthil Eagappan A, Srinivasan D, Natarajan D, Dhanalakshmi V. Effectiveness of parental participation in a dental health program on the oral health status of 8–10-year-old school children. *Int J Clin Pediatr Dent*. 2022;15(4):417–421. doi:10.5005/JP-JOURNALS-10005-2411
30. Toumba KJ, Twetman S, Splieth C, Parnell C, van Loveren C, Lygidakis NA. Guidelines on the use of fluoride for caries prevention in children: An updated EAPD policy document. *Eur Arch Paediatr Dent*. 2019;20(6):507–516. doi:10.1007/s40368-019-00464-2
31. Ihab M, Abdelaziz WEED, Hassan W, El Tantawi M. Development and acceptability of behavioral interventions promoting mothers' brushing of pre-school children's teeth: The preparation phase of the multi-phase optimization strategy framework. *BMC Oral Health*. 2023;23(1):616. doi:10.1186/s12903-023-03351-x
32. Gargano L, Mason MK, Northridge ME. Advancing oral health equity through school-based oral health programs: An ecological model and review. *Front Public Health*. 2019;7:359. doi:10.3389/fpubh.2019.00359
33. Gil GS, Morikava FS, Santin GC, Pintarelli TP, Fraiz FC, Ferreira FM. Reliability of self-reported toothbrushing frequency as an indicator for the assessment of oral hygiene in epidemiological research on caries in adolescents: A cross-sectional study. *BMC Med Res Methodol*. 2015;15:14. doi:10.1186/s12874-015-0002-5

Original paper

# Comparative study of demineralized freeze-dried bone allograft and its combination with platelet-rich fibrin in the treatment of intrabony defects: A randomized clinical trial

Naga Sai Sowparnica Naidu[1,A–F], Anil Kumar Kancharla[1,A,E,F], Anwesh Reddy Nandigam[1,B], Sheema Mohammad Tasneem[1,D], Shiva Shankar Gummaluri[2,E], Swatantrata Dey[1,C], Rini Jyosthna Prathipaty[1,B]

[1] Department of Periodontology, Sree Sai Dental College & Research Institute, Srikakulam, India
[2] Department of Periodontics, GITAM Dental College & Hospital, Vishakapatnam, 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*. 2024;61(4):507–513

**Address for correspondence**
Naga Sai Sowparnica Naidu
E-mail: nagasaisowPi@gmail.com

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on April 1, 2023
Reviewed on May 1, 2023
Accepted on May 16, 2023

Published online on August 9, 2024

## Abstract

**Background.** The clinical and radiographic efficacy of bone grafts and biomaterials, such as platelet-rich plasma and platelet-rich fibrin (PRF), for reconstructing lost periodontal structures has been well documented. However, there is limited data regarding the presence of demineralized freeze-dried bone allograft (DFDBA) in an environment with abundant growth factors provided by platelet concentrates.

**Objectives.** The aim of the study was to compare the clinical and radiographic effectiveness of DFDBA with PRF versus DFDBA alone in the treatment of intrabony defects.

**Material and methods.** Twenty-four intrabony defects in contralateral sites were randomly assigned to either the DFDBA group or the DFDBA combined with PRF group. Clinical parameters, including the plaque index (PI), the gingival index (GI), probing pocket depth (PPD), relative attachment level (RAL), and radiographic bone fill (RBF), were measured at baseline, and at 6 and 9 months. Paired and unpaired *t*-tests were used for intra- and intergroup comparisons.

**Results.** Both the PI and the GI showed statistically significant improvements from baseline to 9 months. However, the intergroup comparisons did not reveal any significant differences ($p < 0.05$) between the groups with regard to clinical and radiographic measurements from baseline to 9 months.

**Conclusions.** Platelet-rich fibrin in combination with DFDBA did not show any additional benefit in terms of reconstructive output in the treatment of intrabony defects compared to the use of DFDBA alone.

**Keywords:** plaque index, gingival index, probing pocket depth, relative attachment level, radiographic bone fill

**Cite as**
Naidu NSS, Kumar Kancharla A, Reddy Nandigam A, et al. Comparative study of demineralized freeze-dried bone allograft and its combination with platelet-rich fibrin in the treatment of intrabony defects: A randomized clinical trial. *Dent Med Probl.* 2024;61(4):507–513. doi:10.17219/dmp/166229

**DOI**
10.17219/dmp/166229

# Introduction

Periodontal disease is inflammatory and polymicrobial in nature. It causes irreversible destruction of the tooth-supporting tissues.[1] Conventional periodontal therapy aims to resolve inflammation and increase the clinical attachment level through repair.[2] Over the past 5 decades, various modalities of treatment have been examined to increase the rate of reconstruction and meet the functional needs of the patient.[3] Autogenous bone grafts have been considered the gold standard for the treatment of intrabony defects. However, these grafts require an additional surgical site and have limitations regarding the quantity of bone that can be harvested in multiple and deep osseous defects.[4] The biologic principles that support the use of autologous and heterologous grafts include not only osteoconductivity and osteoinductivity but also their capacity for space provision and stabilization of blood clots. Demineralized freeze-dried bone allograft (DFDBA) has well-defined properties and has been demonstrated to induce the formation of new bone. The property of osteoinduction is provided by bone morphogenetic proteins (BMPs), which are exposed on the surface of the graft as a result of demineralization. The major BMPs involved in osteoinduction are 2, 4 and 7.[5] Platelet concentrates are considered primers of the coagulation cascade and a bioactive molecular pool. Recent data suggests that the use of these materials as adjuncts has unique and augmented effects on the potential of various reconstructive protocols.[6,7] However, data pertaining to the combination of these materials is not established. Therefore, this study aimed to compare the effectiveness of DFDBA in combination with platelet-rich fibrin (PRF) versus DFDBA alone in the treatment of intrabony defects.

# Material and methods

## Sample size estimation

A sample size of 24 was obtained using G*Power software v. 3.1 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower). The study achieved 80% power with an effect size of 0.25 and an α value of 5%.

## Study design

The study population consisted of subjects who had been referred to the Department of Periodontics (Sree Sai Dental College and Research Institute, Srikakulam, India) for the treatment of periodontitis. The patients were recruited from January 2021 to March 2022. This prospective, single-blind, split-mouth randomized clinical trial included 12 systemically healthy patients, each with at least a pair of contralateral vertical osseous defects (Fig. 1).

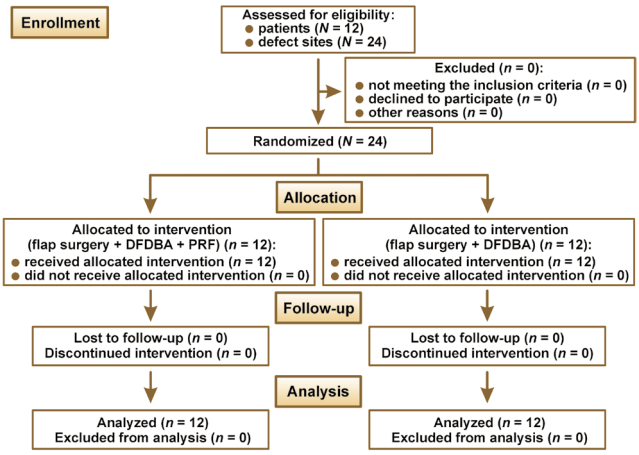


Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flowchart of the study

DFDBA – demineralized freeze-dried bone allograft; PRF – platelet-rich fibrin.

The defects were categorized as two-wall or three-wall defects, according to the classification by Goldman and Cohen.[8] The diagnosis of these defects was based on transgingival probing, which was re-confirmed following surgical exposure. The study was approved by the Sree Sai Dental College & Research Institute Ethical Committee (approval No. SSDC&RI/IRB/IEC/2021-2021/409/8/1) and adhered to the Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines. The study was conducted in accordance with the Declaration of Helsinki, as modified in 2008, and is registered with the Clinical Trials Registry-India (reference No. REF/2021/11/049254). Each participant provided written informed consent after being informed of the benefits and risks associated with the surgical procedure.

## Inclusion criteria

Patients aged between 25 and 55 years, diagnosed with stage III periodontitis (grade A) with a generalized extent and distribution, a minimum of 2 teeth with probing pocket depth (PPD) >6 mm, and angular defects on intraoral periapical radiographs were included in the study.

## Exclusion criteria

Patients with systemic diseases who underwent active periodontal therapy or had a drug history within the previous 6 months, smokers, and pregnant or lactating women were excluded from the study.

## Measurement of clinical parameters

The plaque index (PI) and the gingival index (GI) were measured according to Silness & Löe and Löe & Silness,[9] respectively. Probing pocket depth and relative attachment level (RAL) were recorded at baseline, and at 3, 6 and 9 months postoperatively using a University of North

Carolina (UNC)-15 probe (Hu-Friedy Manufacturing, Co., LLC, Chicago, USA). Customized acrylic stents were fabricated from alginate impressions for accurate measurements. Probing pocket depth with the stent was measured from the crest of the gingival margin to the base of the pocket, while RAL was measured from the point marked at the tip of the stent extending along the buccal tooth surface to the gingival margin. Calibration was conducted by a different examiner who was not involved in the surgical procedure. Before measuring the subjects involved in the study, 10 periodontitis patients were selected at random, and the equipment was calibrated twice with a 24-h interval between each calibration. Only values that were reproduced with consistent results within a margin of ±1 mm and at a confidence level of 90% were accepted.[10]

## Measurement of radiographic parameters

Radiographic bone fill (RBF) was recorded at baseline, and at 6 and 9 months. The models were fabricated from alginate impressions, on which customized X-ray positioning stents were prepared. L-shaped self-curing acrylic resin bite blocks were fabricated on the occlusal surface in the premolar–molar region to retain the plastic film holder using the long cone paralleling technique. The radiographic stent was then evaluated in the patient's mouth for adequate retention and stability (Fig. 2). Dentsply Rinn XCP-DS FIT (Dentsply Sirona, Charlotte, USA) with a posterior aiming ring and a radiographic grid of 1 mm × 1 mm was used for assessment. The exposure parameters were maintained at 75 kVp, 6 mA and 0.8 s. The same voltage was used for taking the radiographs, and the contrast of the radiograph was set to a similar numerical value in SOPRO Imaging software (http://www.soprotechnical-support.com) at baseline and follow-up. Geometric errors were reduced by stent fabrication and the use of the long cone extension technique for both baseline and follow-up radiographs. The amount of bone formed was assessed by comparing the baseline and 9-month radiographs and drawing 2 horizontal lines at the cementoenamel junction (CEJ) and at the alveolar crest, respectively, using the adjacent tooth as a reference (Fig. 3,4). Radiographic bone fill was measured from the CEJ to the base of the defect using SOPRO Imaging software.

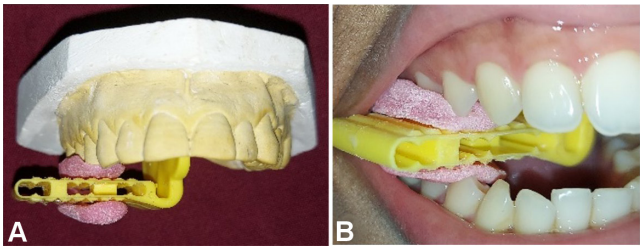


**Fig. 2.** Fabrication of an acrylic stent for the standardization of radiographs
A. Acrylic stent fabricated on the cast model; B. Placement of the stent in the oral cavity.

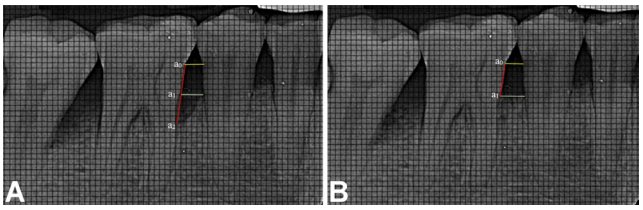


**Fig. 3.** Radiographic evaluation of Site A
A. Baseline; B. 9 months.
$a_0$ – cementoenamel junction; $a_1$ – alveolar crest; $a_2$ – base of the defect.

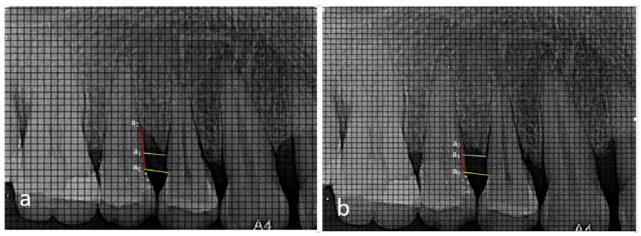


**Fig. 4.** Radiographic evaluation of Site B
A. Baseline; B. 9 months.
$a_0$ – cementoenamel junction; $a_1$ – alveolar crest; $a_2$ – base of the defect.

## Pre-surgical preparation

The patients were provided with educational and motivational support. Full-mouth supra- and subgingival scaling and root planing procedures were performed under local anesthesia, and oral hygiene instructions were given. The full-mouth periodontal status was reassessed before surgery. Twenty-four intrabony defects were randomized into 2 groups by the clinician using a flip coin method. Site A ($n$ = 12) consisted of open flap debridement + DFDBA, and Site B ($n$ = 12) consisted of open flap debridement + PRF + DFDBA. Demineralized freeze-dried bone allograft with particle sizes ranging from 250 µm to 500 µm was obtained from the Tata Memorial Hospital Tissue Bank, Mumbai, India.

## PRF preparation

Platelet-rich fibrin was prepared according to the protocol developed by Dohan et al.[11] Immediately before the surgical procedure, 10 mL of blood was drawn from the subject's antecubital vein. The blood sample was collected in sterile glass test tubes without any anticoagulant and immediately centrifuged in a standardized centrifugation machine (Medico Plus laboratory centrifuge; REMI ELEKTROTECHNIK LTD, Maharashtra, India) at approx. 400 g and 3,000 rpm for 10 min. The centrifuged blood mass formed a structured fibrin clot in the center of the tube, situated between the red blood cell layer at the bottom and the acellular plasma at the top. The fibrin clot (PRF) could be easily removed from the tube and shaped as desired, and was used immediately after collection.

## Surgical procedure

After administering local anesthesia buccally and lingually, sulcular and interdental incisions were made using a No. 15 B.P. blade, resulting in the reflection of a full-thickness mucoperiosteal flap. Meticulous debridement of the defect was performed using Gracey Curettes. Platelet-rich fibrin and DFDBA were placed in a sterile dappen dish. The combined treatments were placed and condensed at the defect in Site B, whereas the graft alone was placed in Site A, filling it to an appropriate level. The flaps were then sutured with simple interrupted silk sutures, resulting in primary tension-free wound closure. The surgical site was covered with COE-PAK™ periodontal dressing on the buccal and lingual aspects (Fig. 5).

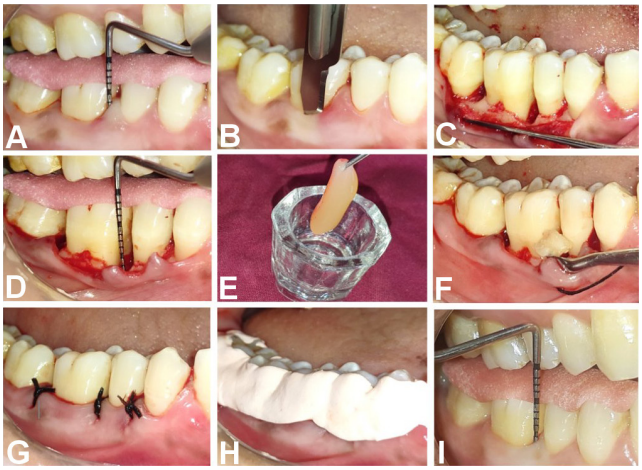


**Fig. 5.** Surgical procedure

A. Measuring the pre-operative probing depth; B. Incision given with No. 15 B.P. blade; C. Full-thickness mucoperiosteal flap reflected; D. Measuring the intrabony defect depth; E. Prepared PRF; F. Placement of DFDBA with PRF into the bony defect; G. Sutures placed; H. Periodontal dressing placed; I. Measuring the post-operative probing depth after 9 months.

## Post-operative care

Patients were instructed to avoid brushing around the surgical site for the first 3 weeks post-surgery. During this period, they were advised to rinse the mouth with a solution of 10 mL of 0.2% chlorhexidine twice daily. A combination of systemic antibiotics (amoxicillin 500 mg, 3 times a day) and analgesics (aceclofenac 100 mg + paracetamol 325 mg, 2 times a day) was prescribed for the 5-day post-surgical period. A soft diet was recommended for a period of 10 days. Patients were also instructed to use minimal pressure when brushing and to use a soft nylon bristle brush for the subsequent 2–3 weeks. Recall appointments were scheduled for pack and suture removal 7 days after surgery. Patients were instructed to report any post-operative discomfort.

## Statistical analysis

In the present study, data was analyzed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). Descriptive statistics and paired *t*-tests were performed for intragroup comparisons, and independent *t*-tests were performed for intergroup comparisons at various time points. The statistician was blinded to the intervention, as both the test and control groups were masked.

# Results

All patients attended the scheduled follow-up visits, and post-operative healing was satisfactory. No post-operative complications were reported. The previously described measurements were recorded accordingly.

Of the 12 patients, 8 (66.7%) were male and 4 (33.3%) were female (Table 1). The mean age of female patients was 42.25 ±8.23 years, while that of male patients was 38.75 ±5.57 years. The overall mean age of the participants was 39.91 ±6.88 years.

**Table 1.** Demographic data of the study participants

| Variable | | DFDBA | DFDBA + PRF | Total |
|---|---|---|---|---|
| Study participants, *N* | | 12 | 12 | 12 |
| Sex | male, *n* | 8 | 8 | 8 |
| | female, *n* | 4 | 4 | 4 |
| Treated premolars and molars | maxillary, *n* | 4 | 5 | 9 |
| | mandibular, *n* | 8 | 7 | 15 |
| Total osseous defects, *n* | | 12 | 12 | 24 |

DFDBA – demineralized freeze-dried bone allograft; PRF – platelet-rich fibrin.

A statistically significant reduction in mean values was observed for both the PI and the GI from baseline to 9 months ($p < 0.01$), indicating good oral hygiene maintenance among the participants of the study (Table 2).

In intra- and intergroup comparisons, both groups showed a reduction in mean PPD from baseline to 9 months, but this did not reach statistical significance ($p = 0.780$).

At Sites A and B, there was a statistically significant reduction in mean RAL from baseline to 9 months ($p < 0.01$). However, in the intergroup comparison, no statistically significant differences were observed between the groups at different time points.

For RBF, both groups showed a significant reduction from baseline to 9 months ($p = 0.002$). However, there were no statistically significant differences between the groups (Table 3).

**Table 2.** Intergroup comparison of mean differences for various parameters at baseline, 3 months, 6 months, and 9 months

| Clinical parameter | Site | Baseline *M* ±*SD* | 3 months *M* ±*SD* | 6 months *M* ±*SD* | 9 months *M* ±*SD* | Intergroup comparison (*p*-value) | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | 3 months | 6 months | 9 months |
| PI | A&B | 1.52 ±0.61 | 0.59 ±0.21 | 0.45 ±0.24 | 0.42 ±0.37 | <0.01* | <0.01* | <0.01* |
| GI | A&B | 2.04 ±1.05 | 0.32 ±0.40 | 0.235 ±0.25 | 0.23 ±0.22 | <0.01* | <0.01* | <0.01* |
| PPD [mm] | A | 6.67 ±0.74 | 2.83 ±0.44 | 2.75 ±0.33 | 2.67 ±0.58 | 0.496 | 0.670 | 0.780 |
| | B | 7.75 ±1.21 | 3.33 ±0.49 | 3.41 ±0.99 | 3.12 ±0.11 | | | |
| RAL [mm] | A | 9.75 ±0.00 | 6.41 ±0.81 | 6.41 ±0.31 | 6.89 ±0.63 | 0.258 | 0.300 | 0.333 |
| | B | 12.75 ±0.00 | 8.75 ±0.00 | 8.83 ±0.06 | 8.90 ±0.22 | | | |

* statistically significant ($p < 0.01$, independent *t*-test); PI – plaque index; GI – gingival index; PPD – probing pocket depth; RAL – relative attachment level; *M* – mean; *SD* – standard deviation.

**Table 3.** Intergroup comparison of mean differences for radiographic bone fill (RBF) at baseline, 6 months and 9 months

| Radiographic parameter | Site | Baseline *M* ±*SD* | 6 months *M* ±*SD* | 9 months *M* ±*SD* | Intergroup comparison (*p*-value) | |
|---|---|---|---|---|---|---|
| | | | | | 6 months | 9 months |
| RBF [mm] | A | 8.19 ±1.01 | 3.71 ±2.40 | 3.22 ±1.63 | 0.412 | 0.819 |
| | B | 7.10 ±2.79 | 3.52 ±1.57 | 3.01 ±2.37 | | |

## Discussion

In this study, DFDBA was used in the control group as it was already established as an effective regenerative material for osseous defects. However, it did not provide any benefit in ridge augmentation due to its rapid resorption rate compared to other grafts, such as FDBA and xenografts. Demineralized freeze-dried bone allograft is a graded material for periodontal regeneration, especially in the treatment of infrabony defects, because of its osteoinductivity.[12,13] This property is attributed to the presence of BMPs.[14–16] The material has passed a series of compliance tests and had its antigenic property removed to ensure its safety for usage.

Platelet concentrates are considered accelerators of soft and hard tissue healing.[17] The quality of the fibrin scaffold in PRF is influenced by a number of factors, including the speed and duration of centrifugation, temperature and blood hematocrit. In this study, PRF was prepared according to the protocol by Dohan et al.[11] Although autografts are the gold standard for treating osseous defects, a study by Mathur et al. found that PRF had comparable efficacy in the treatment of intrabony defects when compared to autogenous bone in the context of three-wall defects.[18] Platelet-rich fibrin demonstrated significantly better results when used alone or in combination with bone grafts. However, the extent of these benefits remained uncertain due to the considerable heterogeneity across the studies and the limited sample size, indicating a low degree of confidence and certainty in the treatment effects.[19–22]

Our study assessed the combined efficacy of DFDBA and PRF compared to DFDBA alone. The study used a split-mouth design, which eliminates selection bias and reduces confounding factors, including environmental, local and systemic influences, as well as prognostic factors, thereby decreasing the heterogeneity.[2] The choice between using a single graft or a combination of grafts and other regenerative materials mainly depends on the selected case and defect morphology. This study evaluated the efficacy of PRF in combination with DFDBA in the treatment of three-wall and two-wall defects, in comparison to DFDBA alone. However, only 2 cases involved two-wall defects, with the remaining cases classified as three-wall defects.

In the present study, plaque control measures were strictly followed, and the patients were highly motivated due to the digital education component. The oral hygiene status was evaluated at the recall visits, and supportive periodontal therapy was provided. Due to the patient's meticulous oral hygiene regimen, there was a decrease in both the PI and GI scores. A reduction in the mean PPD values was observed in both groups at follow-up. These findings are consistent with those reported by Shah et al. and Chadwick et al.[23,24]

Although there were statistically significant differences in RBF from baseline to 9 months in both the DFDBA and DFDBA + PRF groups, no significant differences were observed between the groups. These results are in contrast with studies by Bansal and Bharti and Agarwal et al.[25,26] The 2 main aims of reconstructing a defect are space provision and coinciding of the bone graft resorption time with that of new bone formation. The dimensional stability of PRF has a faster resorption rate and is compromised over time. The proportions of the graft and PRF cannot be controlled when placed in combination, and the graft resorption time does not coincide with PRF. Hence, this may explain the lack of added benefits.

A significant clinical attachment gain was observed in the test group, as evidenced by the active migration of the gingival margin in conjunction with the use of PRF. However, the intergroup comparison did not reach statistically significant results. These findings are in accordance with a study by Nitesh and Anushree.[27] The success and size of attained PRF are contingent upon the timing of blood withdrawal and placement for centrifugation. The optimal timeframe for blood withdrawal is 15 s, with an interval of 60 to 90 s between the drawing of blood and centrifugation recommended to prevent microscopic structural changes.[27]

A limited number of clinical trials have utilized cone-beam computed tomography (CBCT) for accurate measurement of intrabony defects. However, this study attempted to standardize the 2D images by fabricating a special template to increase the accuracy of linear value measurement. Surgical re-entry was not performed for histological evaluations due to ethical considerations. Moreover, the present study did not include a parameter for wound healing, which would have provided valuable insights.[28]

Within the limits of the present study, it can be stated that the use of PRF in conjunction with DFDBA has shown comparable efficacy to DFDBA alone in the treatment of periodontal intrabony defects. Further evaluation through histomorphometric or histologic analysis would have been beneficial for a more comprehensive understanding of the bone regeneration process. With regard to RBF, both treatments showed similar results despite the placement of the graft in a pool of growth factors. Further randomized controlled clinical trials with larger sample sizes and longer follow-up periods are necessary to confirm the beneficial role of PRF in combination with DFDBA in periodontal treatment modalities.

# Conclusions

The present study demonstrated that PRF, when combined with DFDBA, had comparable efficacy to the control group. No additional benefit of PRF was observed. Further randomized clinical trials with larger sample sizes are required to demonstrate the economic benefits and regenerative outcomes of this approach.

## Ethics approval and consent to participate

The study was approved by the Sree Sai Dental College & Research Institute Ethical Committee (approval No. SSDC&RI/IRB/IEC/2021-2021/409/8/1) and adhered to the Consolidated Standards of Reporting Trials (CONSORT) 2010 guidelines. Each participant provided written informed consent after being informed of the benefits and risks associated with the surgical procedure.

## 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

Naga Sai Sowparnica Naidu https://orcid.org/0000-0002-7276-3272
Anil Kumar Kancharla https://orcid.org/0000-0003-2161-0687
Anwesh Reddy Nandigam https://orcid.org/0000-0001-9321-4910
Sheema Mohammad Tasneem https://orcid.org/0000-0002-0044-8302
Shiva Shankar Gummaluri https://orcid.org/0000-0003-3892-7322
Swatantrata Dey https://orcid.org/0000-0002-7666-2495
Rini Jyosthna Prathipaty https://orcid.org/0000-0003-1190-6476


### References


1. Van Dyke TE, Bartold PM, Reynolds EC. The nexus between periodontal inflammation and dysbiosis. *Front Immunol*. 2020;11:511. doi:10.3389/fimmu.2020.00511
2. Needleman I, Tucker R, Giedrys-Leeper E, Worthington H. Guided tissue regeneration for periodontal intrabony defects – A Cochrane Systematic Review. *Periodontol 2000*. 2005;37:106–123. doi:10.1111/j.1600-0757.2004.37101.x
3. Sculean A, Nikolidakis D, Nikou G, Ivanovic A, Chapple ILC, Stavropoulos A. Biomaterials for promoting periodontal regeneration in human intrabony defects: A systematic review. *Periodontol 2000*. 2015;68(1):182–216. doi:10.1111/prd.12086
4. Kumar J, Jain V, Kishore S, Pal H. Journey of bone graft materials in periodontal therapy: A chronological review. *J Dent Allied Sci*. 2016;5:30–34. doi:10.4103/2277-4696.185195
5. Jaiswal Y, Kumar S, Mishra V, Bansal P, Anand KR, Singh S. Efficacy of decalcified freeze-dried bone allograft in the regeneration of small osseous defect: A comparative study. *Natl J Maxillofac Surg*. 2017;8(2):143–148. doi:10.4103/0975-5950.221714
6. Zhou S, Sun C, Huang S, et al. Efficacy of adjunctive bioactive materials in the treatment of periodontal intrabony defects: A systematic review and meta-analysis. *BioMed Res Int*. 2018;2018:8670832. doi:10.1155/2018/8670832
7. Strauss FJ, Nasirzade J, Kargarpoor Z, Stähli A, Gruber R. Effect of platelet-rich fibrin on cell proliferation, migration, differentiation, inflammation, and osteoclastogenesis: A systematic review of in vitro studies. *Clin Oral Investig*. 2020;24(2):569–584. doi:10.1007/s00784-019-03156-9
8. Goldman HM, Cohen DW. The infrabony pocket: Classification and treatment. *J Periodontol*. 1958;29(4):272–291. doi:10.1902/jop.1958.29.4.272
9. Löe H. The gingival index, the plaque index and the retention index systems. *J Periodontol*. 1967;38(6):610–616. doi:10.1902/jop.1967.38.6.610
10. 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
11. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101(3):e37–e44. doi:10.1016/j.tripleo.2005.07.008
12. Randolph RR. *Misch's Contemporary Implant Dentistry*. 4th ed. Los Angeles, CA: Elsevier; 2021.
13. Nasr HF, Aichelmann-Reidy ME, Yukna RA. Bone and bone substitutes. *Periodontol 2000*. 1999;19:74–86. doi:10.1111/j.1600-0757.1999.tb00148.x
14. Urist MR, Strates BS. Bone morphogenetic protein. *J Dent Res*. 1971;50(6):1392–1406. doi:10.1177/00220345710500060601

15. Shigeyama Y, D'Errico JA, Stone R, Somerman MJ. Commercially-prepared allograft material has biological activity in vitro. *J Periodontol*. 1995;66(6):478–487. doi:10.1902/jop.1995.66.6.478
16. Bosshardt DD, Sculean A. Does periodontal tissue regeneration really work? *Periodontol 2000*. 2009;51:208–219. doi:10.1111/j.1600-0757.2009.00317.x
17. Ding ZY, Tan Y, Peng Q, Zuo J, Li N. Novel applications of platelet concentrates in tissue regeneration (review). *Exp Ther Med*. 2021;21(3):226. doi:10.3892/etm.2021.9657
18. Mathur A, Bains VK, Gupta V, Jhingran R, Singh GP. Evaluation of intrabony defects treated with platelet-rich fibrin or autogenous bone graft: A comparative analysis. *Eur J Dent*. 2015;9(1):100–108. doi:10.4103/1305-7456.149653
19. Singh R, Chandrashekar KT, Mishra R, Tripathi VD. Perioglas® and Prf as graft materials in the treatment of intrabony defects in chronic generalized periodontitis: A clinical and radiological evaluation. *J Dent Med Sci*. 2016;15(9):75–81. doi:10.1155/2021/6669168
20. Naqvi A, Gopalakrishnan D, Bhasin MT, Sharma N, Haider K, Martande S. Comparative evaluation of bioactive glass putty and platelet rich fibrin in the treatment of human periodontal intrabony defects: A randomized control trial. *J Clin Diagn Res*. 2017;11(7):ZC09–ZC13. doi:10.7860/JCDR/2017/23831.10149
21. Saravanan D, Rethinam S, Muthu K, Thangapandian A. The combined effect of bioactive glass and platelet-rich fibrin in treating human periodontal intrabony defects – A clinicoradiographic study. *Contemp Clin Dent*. 2019;10(1):110–116. doi:10.4103/ccd.ccd_507_18
22. Panda S, Jayakumar ND, Sankari M, Sankari M, Varghese SS, Kumar DS. Platelet rich fibrin and xenograft in treatment of intrabony defect. *Contemp Clin Dent*. 2014;5(4):550–554. doi:10.4103/0976-237X.142830
23. Shah M, Patel J, Dave D, Shah S. Comparative evaluation of platelet-rich fibrin with demineralized freeze-dried bone allograft in periodontal infrabony defects: A randomized controlled clinical study. *J Indian Soc Periodontol*. 2015;19(1):56–60. doi:10.4103/0972-124X.145803
24. Chadwick JK, Mills MP, Mealey BL. Clinical and radiographic evaluation of demineralized freeze-dried bone allograft versus platelet-rich fibrin for the treatment of periodontal intrabony defects in humans. *J Periodontol*. 2016;87(11):1253–1260. doi:10.1902/jop.2016.160309
25. Bansal C, Bharti V. Evaluation of efficacy of autologous platelet-rich fibrin with demineralized-freeze dried bone allograft in the treatment of periodontal intrabony defects. *J Indian Soc Periodontol*. 2013;17(3):361–366. doi:10.4103/0972-124X.115663
26. Agarwal A, Gupta ND, Jain A. Platelet rich fibrin combined with decalcified freeze-dried bone allograft for the treatment of human intrabony periodontal defects: A randomized split mouth clinical trial. *Acta Odontol Scand*. 2016;74(1):36–43. doi:10.3109/00016357.2015.1035672
27. Nitesh KS, Anushree P. Efficacy of platelet rich fibrin (PRF) in comparison to DFDBA for the treatment of intrabony defect: A randomized controlled clinical study. *Int J Dent Health Sci*. 2016;3(3):470–478. https://nebula.wsimg.com/1f69aaefd4d08f1c039f5d6fdf5e8f09?AccessKeyId=44189AF8BC7E3D5EEFEF&disposition=0&alloworigin=1. Accessed March 30, 2023.
28. Sadek SA, Abbas HM, Alfelali M, Almahdali A. Using acrylic customized X-ray positioning stents for long-term follow-up studies. *Saudi Dent J*. 2020;32(3):120–128. doi:10.1016/j.sdentj.2019.10.001

Original paper

# Exploring the relationship between anxiety, patient characteristics and pain outcomes in oral surgery under local anesthesia: The measurement problem

Ramon Cesar Godoy Gonçalves[A,B,E], Renan Bordini Cardoso[C,E,F], Jessica Bauer[C,E,F], Vitória Mendes dos Santos[B,E,F], Roberto de Oliveira Jabur[A,E,F], Marcelo Carlos Bortoluzzi[A,C,D,F]

Department of Dentistry, State University of Ponta Grossa, Brazil

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(4):515–523

**Address for correspondence**
Marcelo Carlos Bortoluzzi
E-mail: mbortoluzzi@uepg.br

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
We would like to thank the Araucaria Foundation (Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Estado do Paraná, Curitiba, Brazil) for the scholarship granted to Vitória Mendes dos Santos, undergraduate student, as a research assistant.

Received on March 16, 2023
Reviewed on April 1, 2023
Accepted on April 13, 2023

Published online on August 9, 2024

**Cite as**
Godoy Gonçalves RC, Bordini Cardoso R, Bauer J, Mendes dos Santos V, de Oliveira Jabur R, Bortoluzzi MC. Exploring the relationship between anxiety, patient characteristics and pain outcomes in oral surgery under local anesthesia: The measurement problem. *Dent Med Probl.* 2024;61(4):515–523. doi:10.17219/dmp/163255

**DOI**
10.17219/dmp/163255



## Abstract

**Background.** Anxiety during oral surgery can impact patient homeostasis, increase the difficulty of the procedure and create additional stress for the surgeon. Furthermore, it has been associated with more intense and prolonged pain during and after dental treatment.

**Objectives.** The aim of the study is to evaluate the relationship between anxiety, patient characteristics and pain outcomes in oral surgery, as well as to verify the impact of anxiety on patient's perception of pain during and after oral surgery.

**Material and methods.** This is a prospective observational study. Several variables were evaluated during the course of the oral surgery. Anxiety levels were assessed using the State-Trait Anxiety Inventory (STAI), Corah's Dental Anxiety Scale (DAS), the Interval Scale of Anxiety Response (ISAR), and Patient Self-Rated Anxiety (PAnx) during the procedure.

**Results.** General anxiety measures (STAI) were not associated with specific dental anxiety or external observations of anxiety. Anxiety levels varied according to gender and body mass index (BMI), and were correlated with increased heart rate (HR) (with variability among assessment tools). Odontectomy, ostectomy and an increased volume of anesthesia were associated with higher anxiety levels (with variability among the assessment tools). There was a correlation between pain and anxiety, with anxiety contributing to approx. 12% of the variability in postoperative pain.

**Conclusions.** Dental anxiety is a complex, multidimensional mental phenomenon characterized by high variability due to the influence of several dynamic factors.

**Keywords:** pain, dental anxiety, oral surgery, state-trait anxiety inventory

## Introduction

The terms "fear" and "anxiety" are often used interchangeably in dentistry.[1–7] Fear is a normal adaptive response to an immediate threat, whereas anxiety is a response to an anticipated or imagined future threat that can persist and interfere with daily life. However, these terms are usually used synonymously within the field.[1–5,8,9] Individuals who experience fear and anxiety related to dental treatment are often referred to as "dentally anxious patients".[1] Anxiety in dental treatment is defined as an organic response characterized by apprehension and increased surveillance in situations of uncertain danger or potential threats to the organism.[4] There is evidence indicating that individuals with dental anxiety are more likely to have other psychological conditions, such as depression and obsessive-compulsive disorder. Additionally, patients with anxiety and depressive symptoms tend to have poorer oral health.[10,11] Research has also shown that some individuals with dental anxiety may have a history of sexual abuse,[12] highlighting the complexity of the issue.[6]

Oral surgery can elicit pain and a range of negative emotions,[2,3,13–15] with fear of pain during and after oral surgery under local anesthesia being one of the greatest concerns for patients.[2,16] These emotional responses may also affect patient homeostasis[3,15,17–19] and increase the risk of adverse situations, procedural difficulty[8] and stress for the surgeon.[2] In addition, dental anxiety is associated with more intense and prolonged pain during and after dental treatment and oral surgery.[1–5,20–24] Dental anxiety may also exacerbate postoperative symptoms in oral surgery, including speech impairment, altered sensation, appearance concerns, increased pain, sickness, and interference with daily activities.[25] Collectively, these negative emotional experiences lead to increased patient dissatisfaction with dental treatment.[5,9,16]

## Objectives

This is a prospective, exploratory, hypothesis-generating observational study designed to gather information and gain insights about the relationship between anxiety and various variables, conditions and outcomes observed in oral surgery under local anesthesia. The study aims to contribute to the understanding of anxiety by considering a number of factors, including patient characteristics, the type of surgery, procedural techniques, and pain perception during and after oral surgery. The hypothesis states that patient characteristics, the type of surgery and the surgical technique may influence or be influenced by the anxiety levels. Additionally, the study aims to verify the impact of anxiety on patient's perception of pain during and after oral surgery. Improving our understanding of anxiety in oral surgery can lead to the development of improved measurement formats and effective management strategies, resulting in increased patient satisfaction, trust in dentists, and, ultimately, better oral health experiences and outcomes for patients.

## Material and methods

The study was approved by the State University of Ponta Grossa Ethical Committee for Human Research (approval No. 21592119.3.0000.0105/4.383.359) and informed consent was obtained from all participants.

The study population consisted of patients who required oral surgery under local anesthesia. Only patients classified as ASA I or II according to the American Society of Anesthesiologists (ASA) Physical Status Classification System who consented to participate were included in the study. Patients who required premedication with anxiolytics or analgesics (preemptive), pregnant or lactating individuals, minors (under 18 years old), those with contraindications for oral surgery under local anesthesia, as well as patients who developed post-surgical infections or dry sockets were excluded from the analysis. The sample for this exploratory trial was obtained through convenience sampling, with patients included consecutively in a non-random manner. However, for *t*-tests and correlation analysis (point biserial model), the required sample size was calculated a priori using the following input parameters: a two-tailed test; an effect size of $|\rho| = 0.5$; an $\alpha$ error probability of 0.05; and a power (1-$\beta$ err prob) of 0.95, resulting in a minimum sample size of 42 observations or patients (G*Power 3.1.9.7; https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower).

### Clinical anxiety evaluation

To assess patients' anxiety levels, they were asked to complete 2 questionnaires immediately before surgery, namely the State-Trait Anxiety Inventory (STAI) and Corah's Dental Anxiety Scale (DAS). The surgeon and a research assistant completed the Interval Scale of Anxiety Response (ISAR) questionnaire at the end of the procedure. Additionally, patients were requested to evaluate their level of anxiety during the procedure shortly after its conclusion, while still seated in the dental chair.

#### STAI

The State-Trait Anxiety Inventory is a questionnaire designed to evaluate an individual's level of anxiety. The inventory consists of 40 questions, which are divided into 2 subscales: state anxiety (STAI-S); and trait anxiety (STAI-T). The STAI-S reflects a temporary emotional state characterized by feelings of worry and increased activity of the autonomic nervous system. In contrast,

STAI-T indicates a persistent tendency to perceive situations as threatening. Each category is comprised of 20 items, including both direct and reverse-worded questions. Scores range from 20 to 80, with higher scores indicating elevated levels of anxiety. In general, scores between 20 and 40 indicate low anxiety, scores between 41 and 60 indicate moderate anxiety, and scores between 61 and 80 indicate high anxiety.[1,4,9,26,27] To ensure accurate results and eliminate confusion regarding reverse-worded questions, the final scores were calculated using an online calculator (STAI Scoring Tool; https://www.nsrusa.org/score.php).

### DAS

The Brazilian Portuguese version of DAS (or Corah) was used for the assessment of dental anxiety. The questionnaire consists of 4 questions, with responses rated on a scale from 1 (not anxious) to 5 (extremely anxious). Higher scores indicate higher levels of anxiety. Corah's Dental Anxiety Scale assesses the level of anxiety experienced by respondents during dental treatment.[1,23,28] Based on the DAS scores, patients can be classified into 1 of the following categories: (a) low anxiety (4 points); (b) mild anxiety (5–10 points); (c) moderate anxiety (11–15 points); and (d) high anxiety (16–20 points).

### ISAR

The perioperative evaluation of anxiety was conducted from the perspective of the surgeon/research team using the adapted ISAR[1,8,23] immediately after surgery. The questionnaire consists of 10 questions to be rated as observed or not observed based on the following clinical signs: increased perspiration; muscle tension; elevated respiration rate; visible trembling; facial signs of fear or distress; vocal signs such as moaning; self-expression of fear or anxiety by the patient; questions from the patient about treatment necessity or pain; interruption of the procedure by the patient; and the surgeon's assessment of patient's anxiety (no/yes). The adapted version employed a dichotomous scoring system for the signs of anxiety (absent: score 0; present: score 1). The scores from the 10 questions were summed to form a clinical observation score for anxiety during dental surgery, with a range of 0 to 10.

### Perioperative PAnx and pain

Perioperative Patient Self-Rated Anxiety (PAnx) and the pain experienced during surgery were recorded immediately after the surgery. Patients were instructed to rate their anxiety experienced during the procedure using a visual scale ranging from 0 (no anxiety) to 10 (very anxious), as well as to rate the pain experienced under anesthesia (Panes) and the pain during surgery (Psurg) using a visual scale ranging from 0 (no pain) to 10 (severe pain).[23]

## Clinical course of the surgery

The clinical course of oral surgery was recorded, and the following details were collected: the type of surgery (categorized as non-complicated dental extraction, single third molar surgery, or other dentoalveolar/soft tissue oral surgery); the duration of surgery; the necessity for bone surgery; the need for odontectomy; and the amount of anesthesia administered, among others. Additionally, heart rate (HR) was recorded at 5 time points: measurement 1 (m1; pre-surgery in the waiting room); measurement 2 (m2; pre-surgery in the dentist chair and before the wearing of sterile surgical sheets); measurement 3 (m3; during surgery under anesthesia); measurement 4 (m4; during surgery, approximately midway through the procedure); and measurement 5 (m5; immediately after surgery and after the removal of the surgical sheets). Due to the exploratory nature of this study and ethical considerations, all patients were provided with adequate pain control medication and were allowed to discontinue it if they did not experience pain.

## Postoperative pain diary

The patient received a postoperative record. The intensity of pain was self-rated using a visual analog scale (0–100) 7 times (P1 to P7) over a three-day period: at 3 h (P1), 6 h (P2) and 12 h (P3) post-surgery on day 0, and upon waking and at the end of the day (standardized between 6 pm and 8 pm) on days 1 and 2.[24] The patients recorded pain based on the most severe experience between the previous annotations.

## Statistical analysis

The JASP software v. 0.17.1 (https://jasp-stats.org) and/or the JAMOVI Desktop software v. 2.3 (https://www.jamovi.org/download.html) were used to analyze the data through descriptive and inferential methods. A two-tailed probability of $p \leq 0.05$ was considered statistically significant. The variables were categorized as continuous, ordinal, or nominal, and appropriate statistical tests were selected based on these characteristics. The normal or non-normal distribution of the variables was also taken into account, as determined by assumption checks. Missing data was replaced with the sample mean, and outliers were addressed through winsorizing. Cases with missing sensitive data or non-returned diaries were excluded from the analysis.

# Results

The sample comprised 74 surgical procedures performed under local anesthesia. Five cases were excluded due to alveolar infection or dry socket, which could

significantly increase the pain reports. Additionally, 4 cases were excluded due to patients not returning the postoperative pain diary. Of the 65 patients included in the analysis, 40 were female (61%), with a mean age of 30 ±13 years. The mean body mass index (BMI) was 25.2 ±4.6, and the mean years of education was 12 ±2.6. Of the sample, 7 patients (10%) were classified as having a non-restrictive disease (ASA II), while 2 (3%) were current smokers.

Table 1 shows the descriptive statistics for anxiety and pain measurements.

**Table 1.** Descriptive values for the anxiety and pain measurements from day 0 to day 2

| Variable | | *Me* | *M* | *SD* |
|---|---|---|---|---|
| DAS (scale: 4–20) | | 9 | 9.0 | 3.4 |
| STAI-S (scale: 20–80) | | 41 | 41.2 | 11.1 |
| STAI-T (scale: 20–80) | | 41 | 41.8 | 8.5 |
| ISAR (scale: 0–10) | | 5 | 4.8 | 3.2 |
| PAnx (scale: 0–10) | | 5 | 5.0 | 3.1 |
| Self-rated pain (scale: 0–100) | day 0, post-surgery at 3 h | 30 | 34.0 | 28.7 |
| | day 0, post-surgery at 6 h | 20 | 26.5 | 24.8 |
| | day 0, post-surgery at 12 h | 20 | 25.3 | 22.7 |
| | day 0 (mean of the 3 previous measures) | 26.6 | 28.6 | 22.5 |
| | day 1, waking up | 18 | 22.2 | 24.0 |
| | day 1, at the end of the day | 10 | 21.4 | 24.7 |
| | day 1 (mean of the 2 previous measures) | 15 | 21.8 | 23.9 |
| | day 2, waking up | 10 | 18.7 | 23.9 |
| | day 2, at the end of the day | 5 | 17.1 | 23.3 |
| | day 2 (mean of the 2 previous measures) | 10 | 20.2 | 23.9 |
| | mean of all pain measurements | 17.1 | 23.6 | 21.1 |
| Panes (scale: 0–10) | | 2 | 3.0 | 2.6 |
| Psurg (scale: 0–10) | | 1 | 2.0 | 2.5 |

DAS – Corah's Dental Anxiety Scale; STAI-S – State-Trait Anxiety Inventory-state anxiety; STAI-T – State-Trait Anxiety Inventory-trait anxiety; ISAR – Interval Scale of Anxiety Response; PAnx – Patient Self-Rated Anxiety; Panes – pain under anesthesia; Psurg – pain during the surgery; *Me* – median; *M* – mean; *SD* – standard deviation.

## Relationship between anxiety scores

The relationships between the questionnaires and scales used for the assessment of anxiety are shown in Table 2. An exploratory factor analysis, which included the 5 anxiety measurements described above, indicated a clear distinction between the STAI and the other anxiety measurements based on the factor loadings. Following the theoretical principle of differences between the questionnaires, a confirmatory factor analysis was performed, considering 3 factors: (1) generic anxiety (STAI-S and STAI-T); (2) specific dental anxiety (DAS and PAnx); and (3) external anxiety perception (ISAR). The hypothetical model was confirmed as valid ($\chi^2$ = 0.37; comparative fit index (CFI) = 0.99; Tucker–Lewis index (TLI) = 0.99; root mean square error of approximation (RMSEA) = 0.02), indicating that the generic anxiety factor did not show an association with specific dental anxiety ($p$ = 0.52) or external anxiety perception ($p$ = 0.49). However, a significant association was observed between specific dental anxiety and external anxiety perception ($p$ < 0.001).

## Anxiety and demographic variables

The general health status (ASA I or II) of the patient, age, education, and the smoking status did not show any associations or differences regarding the 5 anxiety measurements. A negative correlation was observed between the BMI and both ISAR (Spearman's correlation, rs = –0.3, $p$ = 0.01) and PAnx (Spearman's correlation, rs = –0.26, $p$ = 0.03). Female patients showed higher anxiety scores than males for DAS (Mann–Whitney, $p$ = 0.001) and PAnx (Mann–Whitney, $p$ = 0.01).

## Anxiety and surgical procedures

The increase in HR during surgical procedures showed correlations with ISAR (Spearman's correlation; HRm3 rs = 0.25, $p$ = 0.04; HRm4 rs = 0.29, $p$ = 0.02; HRm5 rs = 0.25, $p$ = 0.04) and with 1 early postoperative

**Table 2.** Correlation between the questionnaires (DAS, STAI and ISAR) and the scales (PAnx) used to evaluate anxiety levels

| Anxiety measurement technique | Spearman's correlation (two-sided) | DAS | STAI-S | STAI-T | ISAR |
|---|---|---|---|---|---|
| STAI-S | Spearman's rho | 0.15 | – | – | – |
| | *p*-value | NS | – | – | – |
| STAI-T | Spearman's rho | 0.25 | 0.58 | – | – |
| | *p*-value | 0.043* | <0.001*** | – | – |
| ISAR | Spearman's rho | 0.28 | 0.14 | –0.1 | – |
| | *p*-value | 0.024* | NS | NS | – |
| PAnx | Spearman's rho | 0.43 | 0.15 | 0.19 | 0.45 |
| | *p*-value | <0.001*** | NS | NS | <0.001*** |

* $p$ < 0.05; *** $p$ < 0.001; NS – non-significant (Shapiro–Wilk test for multivariate normality).

measurement for STAI-T (Spearman's correlation; HRm5 rs = 0.3, $p$ = 0.01). No correlation was observed between HR and DAS, STAI-S and PAnx.

The surgical procedures were classified as follows: 14 (21.5%) were non-complicated dental extractions, 41 (63.1%) were single third molar surgeries and 10 (15.5%) were other dentoalveolar/soft tissue oral surgeries. In the preoperative analysis (DAS, STAI), anxiety levels did not vary significantly (analysis of variance (ANOVA)) according to the type of surgery. However, there were notable differences in anxiety levels during the procedure for ISAR, with third molar surgeries showing higher scores (Levene's $p$-value = 0.8; ANOVA $p$-Tukey < 0.001). No significant differences were observed for PAnx. The surgical time was longer for third molar surgeries, although no statistically significant difference was observed in comparison to other surgical procedures (ANOVA). The reports of patients interrupting surgery due to anxiety were only associated with ISAR (Mann–Whitney, $p$ < 0.001) and PAnx (Mann–Whitney, $p$ < 0.001).

With regard to additional surgical factors associated with anxiety, only the intraoperative scores were relevant for subsequent analysis. The surgical time did not correlate with increased intraoperative anxiety (ISAR and PAnx). The need for osteotomy increased the reported anxiety levels (ISAR, Mann–Whitney, $p$ = 0.01; PAnx, Mann–Whitney, $p$ = 0.037), as did the need for odontectomy (ISAR, Mann–Whitney, $p$ < 0.001). A positive correlation was observed between the increased anesthetic volume and increased surgical anxiety (ISAR, Spearman's correlation, rs = 0.43, $p$ < 0.001; PAnx, Spearman's correlation, rs = 0.28, $p$ = 0.02).

## Anxiety and pain

Greater complaints of pain during anesthesia (Panes) were found to be correlated with PAnx (Spearman's correlation, rs = 0.26, $p$ = 0.03). Anxiety was associated with an increase in pain during surgery (Psurg) (STAI-S, Spearman's correlation, rs = 0.37, $p$ = 0.002; STAI-T, Spearman's correlation, rs = 0.27, $p$ = 0.02; PAnx, Spearman's correlation, rs = 0.43, $p$ < 0.001).

For postoperative pain, anxiety scores measured by DAS, ISAR and PAnx did not show any correlation across the 7 pain measurements. However, STAI-S demonstrated positive and significant correlations (Spearman's correlation) from P2 to P7 (P2, rs = 0.28, $p$ = 0.02; P3, rs = 0.31, $p$ = 0.01; P4, rs = 0.28, $p$ = 0.02; P5, rs = 0.33, $p$ = 0.008; P6, rs = 0.35, $p$ = 0.004; P7, rs = 0.36, $p$ = 0.003). Additionally, STAI-T demonstrated positive and significant correlations (Spearman's correlation) for P3 (rs = 0.25, $p$ = 0.04), P4 (rs = 0.3, $p$ = 0.01), P6 (rs = 0.37, $p$ = 0.002), and P7 (rs = 0.33, $p$ = 0.007). When analyzing the overall mean of pain (mean of all 7 pain measurements) (Table 1), only STAI-S maintained a significant correlation (Spearman's correlation, rs = 0.31, $p$ = 0.01).

To better understand the implications of anxiety on postoperative pain, a path analysis model was created considering the set of 5 anxiety measurements (DAS, STAI-S, STAI-T, ISAR, and PAnx) as exogenous variables and the mean score of all 7 pain measurements as the endogenous variable (Fig. 1). The model was over-identified, and the fit indexes were as follows: $\chi^2$ test $p$-value = 0.1; CFI = 1; adjusted goodness-of-fit index (adj. GFI) = 1; and RMSEA = 0.00. None of the 5 anxiety variables was significantly associated with the mean pain scores. However, the most relevant measurements with greater beta coefficients were ISAR and STAI-S. The model demonstrated that anxiety, as a set of variables, may account for approx. 12% of the variability in pain around the mean value ($R^2$ = 0.12; confidence interval ($CI$) = 0.012–0.290).

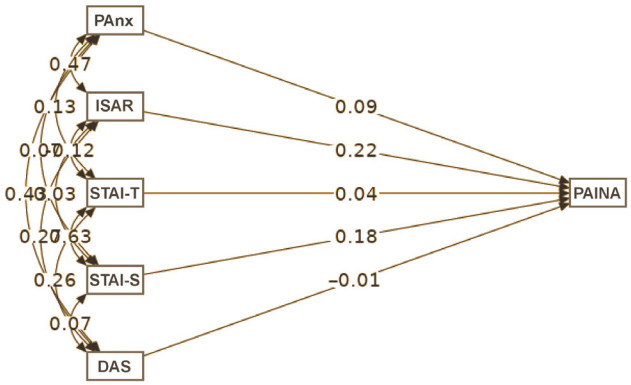


Fig. 1. Relative importance (beta coefficients) of the anxiety measurements on the mean scoring of all 7 pain measurements (PAINA)

No isolated variable was statistically associated with pain in the model, as determined by path analysis using the JAMOVI module.
PAnx – Patient Self-Rated Anxiety; ISAR – Interval Scale of Anxiety Response; STAI-T – State-Trait Anxiety Inventory-trait anxiety; STAI-S – State-Trait Anxiety Inventory-state anxiety; DAS – Corah's Dental Anxiety Scale.

# Discussion

In this study, anxiety was assessed through a series of evaluations conducted during the perioperative period for dental surgical treatment. Comparisons were made between the patient's persistent tendency to perceive situations as threatening (STAI-T), the expectations of anxiety for the surgery (DAS) and the patient's emotional state (STAI-S), which was marked by feelings of worry about what was to come. These variables were compared with the patient's self-reported anxiety levels (PAnx) during the procedure, as well as the observable behavior of the patient's anxiety, as documented by the operators and the research team (ISAR). These comparisons revealed that there was a weak association between DAS and STAI-T, but no association between DAS and STAI-S. In other words, the patient's anxiety profile (STAI-T) influenced their expectations for the procedure (DAS), but these expectations did not influence their current state of anxiety (STAI-S).

However, the patient's profile (STAI-T) strongly influenced their current state of anxiety (STAI-S).

The observed complexity may be attributed to the characteristics of the questionnaires and the timing of their administration. While STAI is a general instrument for evaluating anxiety, DAS is more specific and focused on dental treatment itself, exploring how a patient anticipates feeling during dental procedures. Lin et al.[4] attempted to clarify these distinctions by separating anxiety in dental treatment into state anxiety, which refers to the present moment, and dental care-related anxiety, which refers to a more cognitively involved emotional response to stimuli or experiences associated with dental treatment. However, their meta-analytical study did not identify significant differences between these concepts.[4]

Dental anxiety can also be a fluctuating emotional phenomenon that varies during the course of oral surgery,[23] and that is similar to the physiological responses observed during surgical stress, such as HR.[3] Our findings align with those by Hollander et al.,[3] who observed a correlation between an increase in HR and increased anxiety. However, these results were consistently captured only by ISAR.

It is also necessary to consider the nature of the analysis itself in order to understand dental anxiety. Initially, the statistical procedures used to describe the associations were relatively simple, and associations between DAS and STAI disappeared when gender and/or the type of surgical treatment were included or controlled for in partial correlations (although this was not reported in the main results). Furthermore, the use of more advanced methods, such as Bayesian statistics, might result in the disappearance of the association between DAS and STAI.

Regarding confirmatory factor analysis, to our knowledge, no previous study has explored this set of anxiety measures as different dimensions. The results of our analysis clearly showed that these tools capture different aspects of cognition or understanding related to dental anxiety. The proposed conceptual model suggests 3 different dimensions: general anxiety (STAI-S and STAI-T); specific dental anxiety (DAS and PAnx); and external anxiety perception (ISAR).

Our findings regarding the associations between anxiety measurements were comparable to those observed by Heaton et al.,[1] except for the comparison between DAS and STAI-S. However, it is important to consider the methodological differences between both studies. The abovementioned study reported that DAS was also associated with ISAR (referred to as VAS in their study),[1] similar to our observations. Interestingly, the mean STAI scores observed in the study by Heaton et al.[1] were lower than those obtained in our study, which focused solely on surgical procedures. This difference may be attributed to variations in perceived threats, such as pain and associated trauma, or it may reflect cultural differences among the studied populations. Nevertheless, Heaton et al.[1] also observed that subjects scheduled for surgery obtained higher STAI-S scores, but not other anxiety measures. Similarly, our study found that differences may arise even within the context of proposed surgical treatments. However, this variation was noted only by ISAR (or the team view) and was not reflected in patient perspectives (DAS, STAI and PAnx).

The results of this study indicate that there is no correlation between patient's self-perception of anxiety (PAnx) during surgery and STAI scores. However, a moderate correlation was observed between PAnx and both DAS and ISAR. This raises several questions: Does STAI effectively capture specific dental anxiety? Is the lack of association between STAI and the patient's self-perceived anxiety (PAnx) a problem? Could it be related to the patient's perception of the surgery and self-soothing techniques? Could it be due to the surgeon's (and team's) ability to guide the patient through the surgery? To what extent does the surgical team (and researchers) influence patient responses? To what extent can anxiety and pain measurements be influenced by the Hawthorne effect, and how can this be identified, considered and controlled during data analysis? Could surgical complications or an increased perception of difficulty significantly increase negative emotions in patients? Could the experience of pain during the surgical procedure increase anxiety, or vice versa, creating a feedback loop? To what extent does the subjectivity of pain influence emotional subjectivity?

There are many questions that may not have easy answers, because the subjectivity of emotions, including future perspective threat and surgery threat, meets the real physical trauma and inflammatory responses, intertwining psychology and physiology and highlighting the complexity that may arise from these observations. The limitations of questionnaire-based assessments are apparent, given the considerable gaps in the existing paradigm for health measurements. This underscores the need for substantial updates to these instruments to match the complexity of human emotions. Emerging brain–computer interface technologies may help in a better interpretation of human emotional variability in real-time during surgical procedures, potentially rendering long and inefficient questionnaires obsolete.[23]

In a large systematic review of the factors associated with anxiety related to dental extractions, Astramskaitė et al. observed some similarities among studies and identified problems related to anxiety measurement.[2] The review revealed discrepancies in the correlations between factors and anxiety across studies, which were attributed to the use of different measurement scales. The authors attributed some unreliable study results to measurement issues rather than to deficiencies in the instruments themselves.[2] They suggested that these instruments may not capture the complex array of variables contributing to dental anxiety, including effects, interferences, circumstances, and the intricate psychological pathways of patients.

In summary, the results presented by Astramskaitė et al. showed similarities with our study, such as different anxiety correlations for the same variables depending on the scale used.[2] For example, females showed higher anxiety levels (although with conflicting results), and there were varying findings regarding the association between anxiety levels and education levels and age. Similarly, conflicting results were found regarding anxiety levels and the duration of the procedure, as well as anxiety levels among patients undergoing different surgical treatments. The aforementioned authors noted no association between anxiety and the number of anesthetic injections,[2] whereas our study indicated that increased anxiety was associated with a higher amount of anesthetics used (only with ISAR). Additionally, the authors reported no association between the extent of surgery and increased anxiety.[2] However, we presume that more invasive, longer and challenging procedures, such as odontectomy and ostectomy, may result in higher anxiety levels, which aligns with the observations made in our study.

Aznar-Arasa et al.[8] evaluated dental anxiety in a prospective cohort study focusing on the extraction of impacted lower third molars. They identified correlations between DAS, STAI and ISAR, similar to our study, except for the relationship between STAI with ISAR. The authors observed that procedures involving bone removal and tooth sectioning correlated with higher preoperative anxiety levels, which aligns with our findings.[8] Additionally, they reported that anxious patients experienced significantly more difficulties during lower third molar extractions, a result analogous to our finding that patients who interrupted the surgery most frequently were significantly more anxious (ISAR and PAnx).

Few studies have focused on the relationship between dental anxiety and patient sociodemographic characteristics.[2,9,29] Doganer et al. observed that patients with higher levels of education exhibited lower levels of dental anxiety (STAI-T).[9] Additionally, they found that anxiety levels were higher among patients over 25 years of age, those who had fewer dental visits, and those with a history of dental problems. These findings are consistent with those of other studies.[29,30] Astramskaitė et al. added that marital status and social class might influence different levels of dental anxiety.[2] Our study contributes to these findings by showing that general health status (ASA I and II), age, education, and smoking did not demonstrate any significant associations with the 5 anxiety measurements. However, our findings indicate that anxiety levels may vary according to sex and patients' BMI.

Several studies have shown a correlation between pain and dental anxiety, suggesting that patients with dental anxiety may experience more intense and prolonged pain during and after oral surgery.[1–4,20–24] Our results support previous findings, indicating an association between dental anxiety and increased patient complaints of pain during and after oral surgery, although not with all measurement models used. For example, pain during anesthesia (Panes) was found to correlate only with the patient's self-assessment of anxiety (Panx), whereas the perception of pain during the procedure (Psurg) was associated with STAI (STAI-S and STAI-T) and PAnx. Only STAI demonstrated a correlation with postoperative pain. Despite these associations, the observed patterns suggest the existence of a missing link. If this association is consistent across many observations, it is somewhat surprising that it does not appear consistently across all anxiety measurement instruments.

Our previous study observed a weak but consistent correlation between postoperative pain and higher levels of anxiety (STAI-S), regardless of the number of third molars extracted in a single procedure or the use of pain management medication.[24] In a preliminary report, de Oliveira Jabur et al. observed a significant correlation between postoperative pain and increased levels of anxiety (STAI-T and patient self-evaluation of anxiety),[23] consistent with findings by Lago-Méndez et al.[22] However, as noted by other authors,[2,17] the cumulative results regarding dental anxiety and various factors are inconsistent and controversial. Le et al. argue that existing surveys have significant limitations that may restrict the understanding of patient psychology.[17] They suggest that many psychological assessments in dentistry lack a strong theoretical foundation and were developed for general assessment rather than for specific dental treatments, thereby oversimplifying the complexity of the issue. Lin et al.[4] highlight that anxiety assessment should be a crucial step not only in managing anxiety for highly dentally anxious patients but also in optimizing pain control for all dental patients. The aim of our path analysis model was to explain postoperative pain through anxiety. However, the findings indicated that no single anxiety measurement was significantly associated with pain responses when evaluated collectively as a mean of 7 measurements. However, anxiety may explain approx. 12% of the variability in pain scores around the mean. These results suggest that dental anxiety is a complex, multidimensional mental construct that is influenced by several dynamic factors. This underscores the need for the development of new research approaches and assessment tools in order to better understand this phenomenon. In the words of physicists, we appear to be confronted with a measurement problem.

## Limitations

This study was aimed at gathering information and generating new hypotheses. It had several limitations, primarily due to its exploratory and observational nature. The study sample was obtained through convenience sampling and consecutively selected in a non-random manner, potentially introducing selection bias. The representativeness of the sample might have been limited, and the findings may have limited generalizability as the study included only patients undergoing oral surgery, with data

characteristics that may differ in other circumstances. Although the data analysis considered variables from different perspectives, isolated them and considered potential confounding factors, we cannot exclude the presence of residual factors that may have affected the results.

# Conclusions

The findings of this prospective observational study highlight the following clinical considerations. There is an underlying dimension or latent factor in dental anxiety that cannot be fully explained by isolated measurements from the questionnaires and scales used (DAS, STAI, ISAR, and PAnx). Many correlations were inconsistent both among themselves and with the studied variables, indicating a need for improvements in the assessment tools. Generic instruments such as STAI were not associated with dental anxiety or external observations of anxiety. Female patients and those with lower BMI may exhibit higher levels of anxiety related to oral surgery, with variability across assessment tools. The invasiveness of surgery, including factors such as the volume of anesthesia, odontectomy and ostectomy, was associated with increased levels of anxiety during the procedure. Additionally, anxiety levels correlated with elevated HR (with variability across assessment tools), although the type of surgery proposed did not significantly affect anxiety levels. Pain experienced during and after surgery was found to be associated with increased anxiety levels, with variability across assessment tools. Collectively, the 5 anxiety measurements may account for approx. 12% of the variability in pain.

## Ethics approval and consent to participate

The study was approved by the State University of Ponta Grossa Ethical Committee for Human Research (approval No. 21592119.3.0000.0105/4.383.359) and informed consent was obtained from all participants.

## Data availability

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

## Consent for publication

Not applicable.

### ORCID iDs

Ramon Cesar Godoy Gonçalves https://orcid.org/0000-0002-7488-0771
Renan Bordini Cardoso https://orcid.org/0000-0003-2832-9329
Jessica Bauer https://orcid.org/0000-0003-1749-9816
Vitória Mendes dos Santos https://orcid.org/0000-0002-8480-3868
Roberto de Oliveira Jabur https://orcid.org/0000-0002-2026-1748
Marcelo Carlos Bortoluzzi https://orcid.org/0000-0003-2756-5047

## References

1. Heaton LJ, Carlson CR, Smith TA, Baer RA, de Leeuw R. Predicting anxiety during dental treatment using patients' self-reports: Less is more. *J Am Dent Assoc*. 2007;138(2):188–195. doi:10.14219/jada.archive.2007.0135
2. Astramskaitė I, Poškevičius L, Juodžbalys G. Factors determining tooth extraction anxiety and fear in adult dental patients: A systematic review. *Int J Oral Maxillofac Surg*. 2016;45(12):1630–1643. doi:10.1016/j.ijom.2016.06.019
3. Hollander MHJ, Schortinghuis J, Vissink A. Changes in heart rate during third molar surgery. *Int J Oral Maxillofac Surg*. 2016;45(12):1652–1657. doi:10.1016/j.ijom.2016.08.004
4. Lin CS, Wu SY, Yi CA. Association between anxiety and pain in dental treatment: A systematic review and meta-analysis. *J Dent Res*. 2016;96(2):153–162. doi:10.1177/0022034516678168
5. Alkanan SAM, Alhaweri HS, Khalifa GA, Ata SMS. Dental pain perception and emotional changes: On the relationship between dental anxiety and olfaction. *BMC Oral Health*. 2023;23(1):175. doi:10.1186/s12903-023-02864-9
6. Yao J, Carciofo R, Pan L. Rational thinking as a mediator of the relationship between mindfulness and dental anxiety. *Sci Rep*. 2023;13(1):3104. doi:10.1038/s41598-023-28879-4
7. Janthasila N, Keeratisiroj O. Music therapy and aromatherapy on dental anxiety and fear: A randomized controlled trial. *J Dent Sci*. 2023;18(1):203–210. doi:10.1016/j.jds.2022.06.008
8. Aznar-Arasa L, Figueiredo R, Valmaseda-Castellón E, Gay-Escoda C. Patient anxiety and surgical difficulty in impacted lower third molar extractions: A prospective cohort study. *Int J Oral Maxillofac Surg*. 2014;43(9):1131–1136. doi:10.1016/j.ijom.2014.04.005
9. Doganer YC, Aydogan U, Yesil HU, Rohrer JE, Williams MD, Agerter DC. Does the trait anxiety affect the dental fear? *Braz Oral Res*. 2017;31:e36. doi:10.1590/1807-3107BOR-2017.vol31.0036
10. Aldosari M, Helmi M, Kennedy EN, et al. Depression, periodontitis, caries and missing teeth in the USA, NHANES 2009–2014. *Fam Med Community Health*. 2020;8(4):e000583. doi:10.1136/fmch-2020-000583
11. Hajek A, Lieske B, König HH, et al. Oral health, anxiety symptoms and depressive symptoms: Findings from the survey of health, ageing and retirement in Europe. *Psychogeriatrics*. 2023;23(4):571–577. doi:10.1111/psyg.12963
12. Folayan MO, El Tantawi M, Aly NM, et al. Associations between a history of sexual abuse and dental anxiety, caries experience and oral hygiene status among adolescents in sub-urban South West Nigeria. *BMC Oral Health*. 2021;21(1):196. doi:10.1186/s12903-021-01562-8
13. Shabat MA, Bede SY. Effect of the local application of bupivacaine in early pain control following impacted mandibular third molar surgery: A randomized controlled study. *Dent Med Probl*. 2021;58(4):483–488. doi:10.17219/dmp/133664
14. Silva IA, Agnol Júnior CAD, Weissheimer T, Reis So MV, Da Rosa RA. Pharmacological management of anxiety on pain occurrence during root canal treatment: A systematic review. *Eur Endod J*. 2023;8(2):105–113. doi:10.14744/eej.2022.83097
15. Li X, Tian M, Deng Y, She T, Li K. Advantages of sedation with remimazolam compared to midazolam for the removal of impacted tooth in patients with dental anxiety. *J Oral Maxillofac Surg*. 2023;81(5):536–545. doi:10.1016/j.joms.2023.02.001
16. Laskin DM, Carrico CK. What do patients fear most about having oral surgery? *Quintessence Int*. 2019;50(3):204–207. doi:10.3290/j.qi.a41919
17. Le SH, Tonami K, Umemori S, Nguyen LTB, Ngo LTQ, Mataki S. The potential of heart rate variability for exploring dental anxiety in mandibular third molar surgery. *Int J Oral Maxillofac Surg*. 2018;47(6):809–815. doi:10.1016/j.ijom.2018.01.019
18. Shetty S, Dalvi S, Katge F, Patil D, Chimata VK, Shetty A. Comparison of pain perception between computer-controlled local anesthetic delivery and the conventional syringe for inferior alveolar nerve block in children. *Dent Med Probl*. 2022;59(4):523–529. doi:10.17219/dmp/135897
19. Shimoda H, Yamauchi K, Takahashi T. Transient asystole associated with vasovagal reflex in an oral surgery patient: A case report. *SAGE Open Med Case Rep*. 2023;11:2050313X221146019. doi:10.1177/2050313X221146019

20. Wang TF, Wu YT, Tseng CF, Chou C. Associations between dental anxiety and postoperative pain following extraction of horizontally impacted wisdom teeth: A prospective observational study. *Medicine (Baltimore)*. 2017;96(47):e8665. doi:10.1097/MD.0000000000008665
21. Reyes-Gilabert E, Luque-Romero LG, Bejarano-Avila G, Garcia-Palma A, Rollon-Mayordomo A, Infante-Cossio P. Assessment of pre and post-operative anxiety in patients undergoing ambulatory oral surgery in primary care. *Med Oral Patol Oral Cir Bucal*. 2017;22(6):e716–e722. doi:10.4317/medoral.21929
22. Lago-Méndez L, Diniz-Freitas M, Senra-Rivera C, Seoane-Pesqueira G, Gándara-Rey JM, García-García A. Postoperative recovery after removal of a lower third molar: Role of trait and dental anxiety. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;108(6):855–860. doi:10.1016/j.tripleo.2009.07.021
23. de Oliveira Jabur R, Gonçalves RCG, Faria KW, Semczik IM, Ramacciato JC, Bortoluzzi MC. Single-channel electroencephalography and its associations with anxiety and pain during oral surgery: A preliminary report. *J Dent Anesth Pain Med*. 2021;21(2):155–165. doi:10.17245/jdapm.2021.21.2.155
24. Gonçalves RCG, Jabur RO, Faria KW, Semczik IM, Gross DJ, Bortoluzzi MC. Can anxiety in third molar surgeries with different degrees of difficulty and extent interfere with the perception of postoperative pain and trismus? – An observational and prospective study. *Front Oral Maxillofac Med*. 2021;3:25. doi:10.21037/fomm-21-22
25. Qiao F, Zhang M, Zhang T, Zhu D. Dental anxiety is related to postoperative symptoms in third molar surgery. *Front Psychiatry*. 2022;13:956566. doi:10.3389/fpsyt.2022.956566
26. Koga S, Seto M, Moriyama S, Kikuta T. Anxiety before dental surgery under local anesthesia: Reducing the items on state anxiety in the State-Trait Anxiety Inventory-form X. *J Dent Anesth Pain Med*. 2017;17(3):183–190. doi:10.17245/jdapm.2017.17.3.183
27. Sirin Y, Yildirimturk S, Ay N. Do state-trait anxiety and previous unpleasant dental experiences predict the need for sedation in women having third molar surgery? *Br J Oral Maxillofac Surg*. 2020;58(5):530–534. doi:10.1016/j.bjoms.2020.02.004
28. Bonafé FSS, Campos JADB. Validation and invariance of the dental anxiety scale in a Brazilian sample. *Braz Oral Res*. 2016;30(1):e138. doi:10.1590/1807-3107BOR-2016.vol30.0138
29. Badran A, Keraa K, Farghaly MM. The impact of oral health literacy on dental anxiety and utilization of oral health services among dental patients: A cross sectional study. *BMC Oral Health*. 2023;23(1):146. doi:10.1186/s12903-023-02840-3
30. Tarrosh MY, Alhazmi YA, Aljabri MY, et al. A systematic review of cross-sectional studies conducted in the Kingdom of Saudi Arabia on levels of dental anxiety between genders and demographic groups. *Med Sci Monit*. 2022;28:e937470. doi:10.12659/MSM.937470

Original paper

# Fatty infiltration and morphology of cervical muscles in patients with temporomandibular disorders: A case–control study

Besime Ahu Kaynak[1,A–F], Serkan Taş[2,A–F], Muhammed Taha Tüfek[3,B,C,F], Figen Dağ[4,A,B,F], Rumeysa Dikici[5,A–F]

[1] Department of Health Management, Faculty of Health Science, Toros University, Mersin, Turkey
[2] Department of Physical Therapy and Rehabilitation, Faculty of Health Science, Toros University, Mersin, Turkey
[3] Department of Therapy and Rehabilitation, Vocational School of Health Services, Toros University, Mersin, Turkey
[4] Department of Medical Services and Techniques, Vocational School Of Medical Services, Mersin University, Turkey
[5] Department of Anatomy, Faculty of Medicine, Alanya Alaaddin Keykubat University, Alanya, 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*. 2024;61(4):525–532

**Address for correspondence**
Besime Ahu Kaynak
E-mail: ahu.kaynak@toros.edu.tr

**Funding sources**
The study was supported by the Scientific Research Projects Unit of Toros University (grant No. 2019-SBYO-BAK-1).

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on March 15, 2023
Reviewed on May 7, 2023
Accepted on May 17, 2023

Published online on August 9, 2024

**Cite as**
Kaynak BA, Taş S, Tüfek MT, Dağ F, Dikici R. Fatty infiltration and morphology of cervical muscles in patients with temporomandibular disorders: A case–control study. *Dent Med Probl.* 2024;61(4):525–532. doi:10.17219/dmp/166266

**DOI**
10.17219/dmp/166266



## Abstract

**Background.** Changes in the fatty infiltration and/or muscle volume of neck muscles can alter cervical spine alignment and cranial load distribution, which may cause pain in the orofacial region.

**Objectives.** The aim of the study was to examine the muscle volume and fatty infiltration of neck muscles in patients with temporomandibular disorders (TMD).

**Material and methods.** This case–control study included 18 patients with TMD and 18 age- and sex-matched controls. The muscle volume and fatty infiltration of the neck muscles of the participants were measured using magnetic resonance imaging (MRI) and ITK-SNAP software. The 3D models of the sternocleidomastoid (SCM), splenius capitis (SPLC), semispinalis cervicis (SC)–semispinalis capitis (SCP), and multifidus (M) muscles within the C3–C7 range were created using ITK-SNAP, a semi-automatic segmentation software. The models were used to determine the volumes and fatty infiltration levels. The Neck Disability Index (NDI) was used to assess neck pain-related disability. The severity of TMD was determined using the Fonseca Anamnestic Index (FAI), while jaw-related disability was measured with the Jaw Functional Limitation Scale-20 (JFLS-20). Pain levels were recorded at rest and during chewing using the numeric rating scale (NRS).

**Results.** There were no statistically significant differences in total muscle volume, fatty infiltration volume and fatty infiltration percentage of the SCM, SPLC, SCP, SC, and M muscles between the 2 groups ($p > 0.05$). The patient group had higher NDI scores compared to the controls ($p < 0.001$). The NDI scores correlated positively with the JFLS-20 ($r = 0.831$, $p < 0.001$), FAI ($r = 0.815$, $p < 0.001$) and NRS scores at rest ($r = 0.753$, $p < 0.001$) and during chewing ($r = 0.686$, $p < 0.001$).

**Conclusions.** The present study did not identify any significant differences in the neck muscle volume or fatty infiltration between the TMD patients and controls. However, the severity of neck disability was found to correlate with jaw function, pain and TMD levels.

**Keywords:** muscle, volume, temporomandibular disorders, fatty infiltration, neck

# Introduction

Temporomandibular disorders (TMD) are a common group of musculoskeletal conditions, affecting around 31% of the general population and leading to pain and/or dysfunction in the temporomandibular joint, masticatory muscles and related structures.[1–3] The impact of TMD symptoms on an individual's quality of life is comparable to that of low back pain or severe headaches.[4]

Temporomandibular disorders often manifest with symptoms that affect the cervical region, such as neck pain, tender points, cervical spine dysfunction, and changes in cervical spine posture and head posture.[2,5–9] Studies have shown a correlation between orofacial disorders and neck disorders, which can be attributed to physiological, neuronal, biomechanical, and anatomical connections between the craniofacial and cervical spine regions.[2,5–9] Alterations in the cervical spine posture can affect jaw movements and the activation of masticatory muscles due to the existence of muscular and ligamentous connections between the temporomandibular joint and the cervical spine.[10,11] In addition, cervical pain can lead to pain in the orofacial region through reflex and neural connections involving mechanoreceptors and nociceptors within the cervical muscle system and the temporomandibular joint.[12–14] Nociceptive signals originating from the cervical region result in an increase in central sensitization in the trigeminocervical nucleus, thereby causing pain in the orofacial region.[12]

There is extensive evidence of neurological and anatomical connections between the cervical region and TMD. However, there is a dearth of research related to the motor control, endurance and strength of the neck muscles. To date, there have been a limited number of studies investigating the endurance and/or strength of neck muscles in patients with TMD. Previous studies have reported a decrease in the endurance and strength of neck flexor muscles and the endurance of neck extensor muscles in patients with TMD.[5,15–17] However, to the best of our knowledge, no study has investigated the fatty infiltration and muscle volume of neck muscles using magnetic resonance imaging (MRI) in patients with TMD. Muscle volume is a major determinant of muscle force production.[18] Additionally, measuring fatty infiltration provides important information about muscle quality.[19] The cervical muscles play an important role in the maintenance of neutral alignment, cranial load distribution and horizontal gaze during neck motion.[17,20,21] Changes in the fatty infiltration and/or muscle volume of neck muscles could affect the force production of neck muscles[18,19] and alter cervical spine alignment and cranial load distribution, potentially causing orofacial pain.[10,11,20–22] The identification of potential changes in the volume and fatty infiltration of neck muscles in patients with TMD could help clinicians make adequate treatment decisions. Therefore, the aim of this study was to examine muscle fatty infiltration and muscle volume ratio in neck muscles of patients with TMD and to compare these findings with those of asymptomatic participants. It was hypothesized that there would be a reduction in muscle volume and an increase in the fatty infiltration in the neck muscles of patients with TMD in comparison to asymptomatic participants.

# Material and methods

## Sample size calculation

To determine the minimum number of cases to be included in the study, a power analysis was conducted using specialized software (SPSS Sample Power 3.0; IBM Corp., Armonk, USA). The sample size calculation indicated that a minimum of 13 participants per group is required, assuming an alpha level (α) of 0.05 and a desired power (β) of 80% to detect a minimum difference of 6.9% in the fatty infiltration of the multifidus (M) and semispinalis cervicis (SC) muscles. This calculation was based on a mean muscle fatty infiltration of 23% in the control group, with a standard deviation (*SD*) of 6.3%.[23]

## Participants

This case–control study included the staff and students of Toros University (45 Evler Campus), Mersin, Turkey, and was conducted between March 2020 and November 2022. The presence of TMD was evaluated using the Fonseca Anamnesis Index (FAI), which has demonstrated high diagnostic accuracy.[24] Individuals with moderate or severe TMD (FAI score ≥45) and individuals without TMD (FAI score ≤15), according to the FAI, were invited to participate.[25,26] Only patients with moderate and severe TMD were included in the study, because the differences in the examined parameters were expected to be more pronounced. The individuals who agreed to participate were evaluated by an oral surgeon who had received training in the diagnostic criteria for TMD (DC/TMD) and had 25 years of clinical experience. Individuals who met the DC/TMD (Ia) were included in the TMD group. The TMD group consisted of individuals who had experienced pain in the temporomandibular joint and/or masticatory muscles for at least 6 months before the study, with a pain severity rating of at least 3 on the numeric rating scale (NRS). The control group consisted of asymptomatic individuals who had not experienced pain related to the neck region, temporomandibular joint, or masticatory muscles for at least 1 year before the study. At the end of the clinical evaluations, 18 participants with TMD (10 males, 8 females) aged 27–57 years and 18 controls (9 males, 9 females) aged 26–58 years were included in the study (Fig. 1). Participants who reported any of the following were excluded from the study: a history of receiving treatment related to the neck and/or TMD in the past

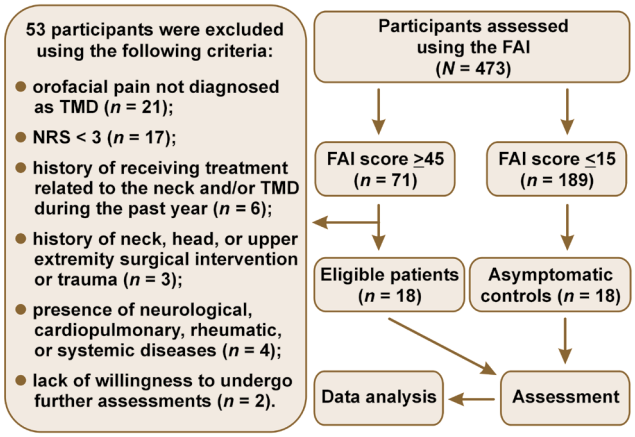


Fig. 1. Flowchart of sample selection process

TMD – temporomandibular disorders; NRS – numeric rating scale; FAI – Fonseca Anamnestic Index.

year; a history of neck, head, or upper extremity surgical intervention or trauma; presence of neurological, cardiopulmonary, rheumatic, or systemic diseases. The study was conducted in accordance with relevant guidelines and regulations, including the Declaration of Helsinki.

## Pain assessment

The severity of pain experienced by the participants was evaluated at rest and during chewing using the NRS, which is a reliable and valid tool for the assessment of pain severity.[27,28] On the NRS, a score of 0 indicates no pain, while a score of 10 represents the worst pain imaginable.

## Assessment of disability and symptom severity

The severity of TMD was determined by the FAI, which has been validated and shown to be reliable in the Turkish-speaking population.[24] The index consists of 10 questions, each offering 3 possible responses: no (0 points); sometimes (5 points); and yes (10 points). The FAI scores range from 0 to 100. The total score is used to classify the severity of TMD as follows: no TMD (≤15); mild TMD (20–40); moderate TMD (45–65); and severe TMD (70–100).[25]

The Jaw Functional Limitation Scale-20 (JFLS-20) was used to evaluate disability related to jaw function. This scale has been validated and proven reliable for evaluating jaw-related disabilities.[29] It consists of 20 questions, with each question scored on a scale of 0 (no limitation) to 10 (severe limitation).

The Neck Disability Index (NDI) was used to determine disability related to neck pain. The index has been validated and shown to be a reliable instrument for assessing neck pain-related disability in the Turkish-speaking population.[30] The scale consists of 10 questions. There are 5 optional answers for each question, ranging from 0 (no disability) to 5 (complete disability).

## MRI measures and analysis

All MRI studies were conducted using a 1.5 Tesla system (Philips Ingenia Ambition MRI system; Philips Healthcare, Best, the Netherlands) with a 16-channel receiver coil. The MRI protocol consisted of a T1-weighted turbo spin echo (TSE) array (repetition time/echo time (TR/TE) = 567/15 ms; TSE factor 3, slice thickness = 3 mm) and a T2-weighted array. The images were acquired with the subject's mouth closed. Axial images were obtained using the T1-weighted MRI protocol. The T2-weighted parasagittal images were acquired using the axial localizer image.

A semi-automatic segmentation software, ITK-SNAP (http://www.itksnap.org/pmwiki/pmwiki.php), was used to generate a 3D model of the sternocleidomastoid (SCM), splenius capitis (SPLC), SC–semispinalis capitis (SCP), and M muscles within the C3–C7 range, as well as to calculate their volumes (Fig. 2,3). The MRI files were converted from the Digital Imaging and Communications in Medicine (DICOM) format to the Neuroimaging Informatics Technology Initiative (NIfTI) format using dcm2niix software (https://people.cas.sc.edu/rorden/mricron/dcm2nii.html).[31] Subsequently, the files were uploaded to ITK-SNAP, where the boundaries of the deep neck muscles and the SCM muscle were delineated in 3D coordinates. For each scan, the boundaries of the muscles were determined using the active contour segmentation method. The muscle body contrast was created using the clustering 3/1 option, and the adipose tissue contrast was created using the clustering 3/2 option.[32,33] Each muscle and adipose tissue region was enclosed in a bubble

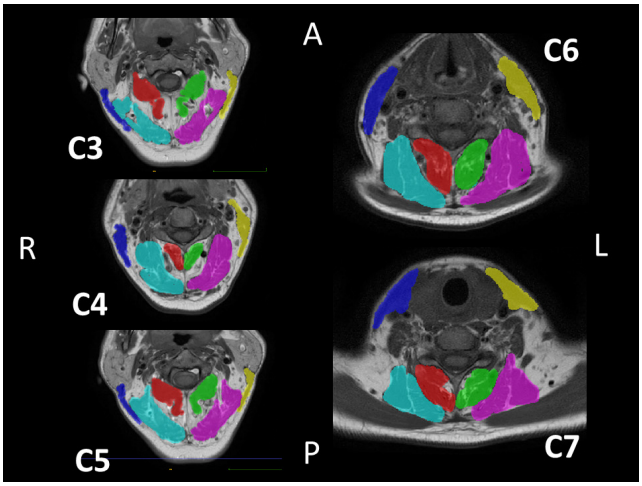


Fig. 2. Magnetic resonance imaging (MRI) data of axial sections of the multifidus (M), semisipinalis cervicis (SC), semispinalis capitis (SCP), splenius capitis (SPLC), and sternocleidomastoid (SCM) muscles in the C3–C7 range marked with various colors using ITK-SNAP software

Each color represents a different muscle/muscle group, as follows: blue – right SCM; turquoise – right SCP+SPLC; red – right M+SC; green – left M+SC; pink – left SCP+SPLC; yellow – left SCM; A – anterior; P – posterior; R – right; L – left.

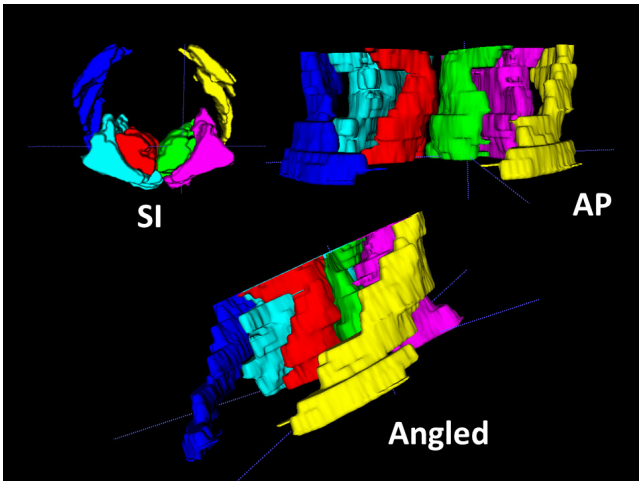


Fig. 3. 3D model of the M, SC–SCP, SPLC, and SCM muscles in the C3–C7 range created using ITK-SNAP software to determine the muscle volume

The anterior-posterior (AP), superior-inferior (SI) and angled view of the model. Each color represents a different muscle/muscle group, as follows: blue – right SCM; turquoise – right SCP+SPLC; red – right M+SC; green – left M+SC; pink – left SCP+SPLC; yellow – left SCM.

created within the ITK-SNAP interface, and the 3D model was filled by determining the processor speed. The volumes were recorded in $mm^3$ from the volumes and statistics tab of the software.[34] All measurements were performed by the same investigator who was blinded to the study groups.

## Statistical analysis

The data was analyzed using the IBM SPSS Statistics for Windows software, v. 22.0 (IBM Corp., Armonk, USA). Visual and analytical methods were used to assess whether the evaluated parameters were normally distributed. As the parameters did not show a normal distribution, the demographic data and the assessed parameters are presented using the median (*M*) (interquartile range (*IQR*)). The Mann–Whitney *U* test was used to compare the differences between the parameters in different groups. The Wilcoxon test was employed to assess differences between the right and left sides in patients with TMD and the control group. Correlation coefficients were calculated using the Spearman's test to determine the relationships between the NDI score and the JFLS-20, FAI and NRS scores in patients with TMD. A $p$-value <0.05 was considered statistically significant.

# Results

No statistically significant differences were observed between the TMD and control groups in terms of age ($p$ = 0.389), height ($p$ = 0.650), weight ($p$ = 0.584), and body mass index (BMI) ($p$ = 0.888). The *M* (*IQR*) for the FAI, JFLS-20, NDI, and NRS scores at rest and during chewing in patients with TMD were 55 (50–65), 40 (28–65), 14 (9–17), 4 (3–5), and 5 (3–6), respectively. The TMD group had higher FAI ($p$ < 0.001), JFLS-20 ($p$ < 0.001) and NDI ($p$ < 0.001) scores compared to the control group (Table 1). There were no significant differences between the groups with regard to the mean total volume of the M+SC, SCP+SPCL and SCM muscles on the left and right sides ($p$ > 0.05). Similarly, no differences were observed between the TMD and control groups with regard to the total fatty infiltration volume and fatty infiltration percentage of the assessed muscles ($p$ > 0.05) (Table 2). In addition, no differences were found between the right and left sides within the TMD group regarding the mean total volume of M+SC ($p$ = 0.500), SCP+SPCL ($p$ = 0.699) and SCM ($p$ = 0.744), as well as the total fatty infiltration volume of M+SC ($p$ = 0.679), SCP+SPCL ($p$ = 0.679) and SCM ($p$ = 0.191). There were no differences between the right and left sides in the control group for the mean total volume of M+SC ($p$ = 0.372), SCP+SPCL ($p$ = 0.408) and SCM ($p$ = 0.586), as well as for the total fatty infiltration volume of M+SC ($p$ = 0.257), SCP+SPCL ($p$ = 0.896) and SCM ($p$ = 0.191).

Correlation analyses revealed a positive correlation between the NDI scores and the JFLS-20 ($r$ = 0.831, $p$ < 0.001), FAI ($r$ = 0.815, $p$ < 0.001) and NRS at rest ($r$ = 0.753, $p$ < 0.001) and during chewing ($r$ = 0.686, $p$ < 0.001) in the TMD group.

Table 1. Demographic parameters of individuals with temporomandibular disorders (TMD) and controls

| Parameter | | Control group ($n$ = 18) *M* (*IQR*) | TMD group ($n$ = 18) *M* (*IQR*) | $p$-value |
|---|---|---|---|---|
| Age [years] | | 38 (32–45) | 41 (34–48) | 0.389 |
| Height [m] | | 1.65 (1.63–1.76) | 1.70 (1.65–1.75) | 0.650 |
| Weight [kg] | | 70 (57–82) | 72 (64–79) | 0.584 |
| BMI [kg/m$^2$] | | 25.1 (21.5–26.7) | 25.0 (22.2–27.7) | 0.888 |
| Sex, *n* (%) | male | 9 (50.0) | 10 (55.6) | – |
| | female | 9 (50.0) | 8 (44.4) | – |
| NRS | pain at rest | 0 | 4 (3–5) | <0.001* |
| | pain when chewing | 0 | 5 (3–6) | <0.001* |
| FAI | | 5 (0–10) | 55 (50–65) | <0.001* |
| JFLS-20 | | 0 (0–7) | 40 (28–65) | <0.001* |
| NDI | | 2 (1–6) | 14 (9–17) | <0.001* |

* statistically significant ($p$ < 0.05, Mann–Whitney *U* test); NRS – numeric rating scale; FAI – Fonseca Anamnestic Index; JFLS-20 – Jaw Functional Limitation Scale-20; NDI – Neck Disability Index; *M* – median; *IQR* – interquartile range; BMI – body mass index.

**Table 2.** Total muscle volume, total fatty infiltration volume and fatty infiltration percentage of the assessed muscles in individuals with TMD and controls

| Parameter | | | Control group ($n$ = 18) *M* (*IQR*) | TMD group ($n$ = 18) *M* (*IQR*) | *p*-value (Mann–Whitney *U* test) |
|---|---|---|---|---|---|
| M+SC | right side | C3–C7 total volume [mm$^3$] | 8,007 (4,871–9,525) | 8,185 (6,000–10,868) | 0.584 |
| | | fatty infiltration [mm$^3$] | 2,288 (1,201–2,874) | 2,251 (1,860–2,652) | 0.628 |
| | | fatty infiltration [%] | 23.5 (21.6–32.4) | 27.8 (19.5–34.1) | 0.719 |
| | left side | C3–C7 total volume [mm$^3$] | 8,571 (4,938–9,314) | 8,123 (6,311–10,221) | 0.521 |
| | | fatty infiltration [mm$^3$] | 2,177 (1,140–2,654) | 2,246 (1,774–2,784) | 0.501 |
| | | fatty infiltration [%] | 24.2 (19.5–31.6) | 27.2 (22–32.1) | 0.389 |
| SCP+SPCL | right side | C3–C7 total volume [mm$^3$] | 9,576 (7,690–12,698) | 11,426 (8,758–12,782) | 0.521 |
| | | fatty infiltration [mm$^3$] | 566 (365–674) | 481 (341–658) | 0.719 |
| | | fatty infiltration [%] | 4.4 (4.1–6.5) | 4.4 (3.1–7.2) | 0.389 |
| | left side | C3–C7 total volume [mm$^3$] | 9,013 (7,366–12,198) | 10,121 (8,359–12,241) | 0.628 |
| | | fatty infiltration [mm$^3$] | 538 (354–584) | 507 (325–688) | 0.888 |
| | | fatty infiltration [%] | 4.5 (3.7–7.9) | 4.8 (2.6–7.6) | 0.815 |
| SCM | right side | C3–C7 total volume [mm$^3$] | 7,671 (5,377–9,912) | 7,464 (6,199–10,874) | 0.606 |
| | | fatty infiltration [mm$^3$] | 408 (214–650) | 439 (244–654) | 0.563 |
| | | fatty infiltration [%] | 4.5 (3.7–5.3) | 5.7 (2.8–8.0) | 0.542 |
| | left side | C3–C7 total volume [mm$^3$] | 7,614 (5,260–9,630) | 7,428 (6,109–10,320) | 0.650 |
| | | fatty infiltration [mm$^3$] | 417 (248–574) | 463 (314–587) | 0.308 |
| | | fatty infiltration [%] | 4.5 (3.7–5.9) | 6.1 (3.5–8.8) | 0.279 |

M – multifidus muscle; SC – semispinalis cervicis muscle; SCP – semispinalis capitis muscle; SPLC – splenius capitis muscle; SCM – sternocleidomastoid muscle.

## Discussion

To the best of our knowledge, this study is the first to investigate changes in the muscle volume and fatty infiltration of neck muscles in patients with TMD. We hypothesized that there would be differences in these parameters between patients with TMD and asymptomatic controls. This hypothesis is based on the premise that reductions in the muscle volume and increases in the fatty infiltration may impair motor function and power generation of neck muscles, potentially affecting the orofacial region due to physiological, neuronal, biomechanical, and anatomical connections between the craniofacial and cervical spine regions.[2,5–9] However, contrary to our hypothesis, we found that the muscle volume and fatty infiltration of the SCM, SPLC, SCP, SC, and M muscles were similar in patients with TMD and controls.

Previous studies have investigated neck muscle function in patients with TMD by evaluating muscle strength or endurance using clinical tests.[5,15,17] These studies have reported a reduction in the endurance of the flexor neck muscles[15] and a decline in the extensor neck muscles in patients with TMD.[5] However, the strength of the extensor neck muscles was found to be similar between TMD patients and controls.[17] Our findings can be compared with those of previous studies investigating fatty infiltration, muscle volume, or muscle cross-sectional area of neck muscles in other musculoskeletal conditions related to the neck, such as cervical myelopathy, neck pain, or whiplash-associated disorders (WAD).

Previous studies have reported greater fatty infiltration in the deep cervical muscles, including the SCP, M, SC, SCM, SPLC, and trapezius muscles in patients with WAD,[35–37] cervical myelopathy,[38] or chronic neck pain.[39,40]

However, the results regarding changes in neck muscle morphology in patients with neck disorders are inconclusive. Some studies have reported a decrease in the cross-sectional area of extensor neck muscles in patients with WAD[37] and chronic neck pain[41,42]; conversely, other studies have indicated an increase in the cross-sectional area of neck muscles in patients with WAD[43] or chronic neck pain.[44] Previous studies have suggested that neck pain can cause changes in muscle morphology and muscle quality. Our results are in contrast with previous findings, possibly due to the level of neck disability observed in patients with TMD in the present study. Our study found higher neck disability scores in patients with TMD compared to controls, although nearly all patients with TMD had mild or moderate neck disability according to the NDI (scores ranged from 6 to 23).[45] In contrast, previous studies have included patients with severe or complete neck disability (NDI > 25), suggesting that the morphology and quality of neck muscles may be affected only in individuals with severe neck disability.

Furthermore, we conducted side-to-side comparisons to determine whether the degree of fatty infiltration and muscle volume of the neck muscles were consistent across patients with TMD and the controls. No significant differences were observed in the fatty infiltration and volume of the neck muscles between the left and right sides in both patient groups. Similar results were reported in previous studies. Specifically, it was found that the fatty infiltration of the cervical extensor muscles in patients with WAD,[36] the cross-sectional area of the extensor neck muscles in patients with WAD,[43] the cross-sectional area of the M muscle in patients with chronic neck pain,[42] and the cross-sectional area and fatty infiltration of the M muscle and short rotators in asymptomatic participants[35] showed no significant differences between the left and right sides.

Similar to previous studies,[46–49] we observed higher neck disability scores in patients with TMD compared to the asymptomatic group. Furthermore, there was a strong correlation between the severity of neck disability and the severity of TMD, jaw-related disability, and pain severity in patients with TMD, indicating an association between neck-related symptoms and TMD-related symptoms. These results suggest that it may be useful to routinely assess neck-related problems in patients with TMD and address these problems in the treatment plan.

Similar to our results, Alves da Costa et al. reported a positive relationship between the severity of neck disability and pain severity in patients with TMD.[46] However, Coskun Benlidayi et al. identified a weak correlation between the severity of neck disability and the TMD pain score.[50] Moreover, Silveira et al. reported a strong correlation between jaw disability and neck disability in patients with TMD.[51] Furthermore, de Abreu Figueirêdo et al. found a moderate positive correlation between TMD severity and the severity of neck disability.[52] In contrast to our results, another study indicated that there was no correlation between the severity of neck disability and TMD pain severity in patients with TMD.[53]

## Limitations

The present study had several limitations. Firstly, it was a cross-sectional study conducted at a single time point, thus causality could not be inferred from the findings. Secondly, nearly all participants with TMD included in this study had mild or moderate neck disability, as indicated by the NDI. The extent of neck muscle involvement may differ across patients with both TMD and severe neck disability. Furthermore, the study did not assess the strength or endurance of the neck muscles. Therefore, more information about the relationship between the cervical spine region and TMD could have been obtained if neck muscle strength or endurance had been examined and the relationship between the neck muscles and/or endurance and neck muscle fatty infiltration and volume had been analyzed. Lastly, the number of cases in this study was insufficient to determine the relationships between MRI results and other clinical outcomes, including the NDI, FAI, NRS, and JFLS-20.

# Conclusions

The results showed no statistically significant differences in the volume of the SCM, SPLC, SCP, SC, and M muscles in the C3–C7 region between the patients with TMD and the controls. Furthermore, the degree of fatty infiltration of these muscles was similar in both groups. However, the TMD patients had higher neck disability scores compared to the control group. Moreover, the severity of neck disability was positively correlated with the severity of jaw-related disability, TMD pain severity and the severity of TMD. These results suggest that the cervical region should be evaluated in patients with TMD, and that interventions targeting this region should be included in TMD treatment programs.

## Ethics approval and consent to participate

The study was approved by the Non-Interventional Clinical Research Ethics Board of Mersin University, Turkey (protocol No. 2019/567). Before the experiment, all subjects were informed about the nature of the study and their written informed consent was obtained.

## 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

Besime Ahu Kaynak https://orcid.org/0000-0001-5874-8802
Serkan Taş https://orcid.org/0000-0001-8268-5485
Muhammed Taha Tüfek https://orcid.org/0000-0002-9356-4096
Figen Dağ https://orcid.org/0000-0002-6836-8050
Rumeysa Dikici https://orcid.org/0000-0003-3144-8740


## References


1. Greene CS. Managing the care of patients with temporomandibular disorders: A new guideline for care. *J Am Dent Assoc*. 2010;141(9):1086–1088. doi:10.14219/jada.archive.2010.0337
2. Ghurye S, McMillan R. Pain-related temporomandibular disorder – Current perspectives and evidence-based management. *Dent Update*. 2015;42(6):533–536,539–542,545–546. doi:10.12968/denu.2015.42.6.533
3. Valesan LF, Da-Cas CD, Réus JC, et al. Prevalence of temporomandibular joint disorders: A systematic review and meta-analysis. *Clin Oral Investig*. 2021;25(2):441–453. doi:10.1007/s00784-020-03710-w
4. Drangsholt M. Epidemiology of pain. In: Crombie I, Linton S, LeResche L, Von Korff M, eds. *Temporomandibular Disorder Pain*. IASP Press; 1999:203–233.
5. Armijo-Olivo S, Silvestre RA, Fuentes JP, et al. Patients with temporomandibular disorders have increased fatigability of the cervical extensor muscles. *Clin J Pain*. 2012;28(1):55–64. doi:10.1097/AJP.0b013e31822019f2
6. Kritsineli M, Shim YS. Malocclusion, body posture, and temporomandibular disorder in children with primary and mixed dentition. *J Clin Pediatr Dent*. 1992;16(2):86–93. PMID:1498052.
7. Sonnesen L, Bakke M, Solow B. Temporomandibular disorders in relation to craniofacial dimensions, head posture and bite force in children selected for orthodontic treatment. *Eur J Orthod*. 2001;23(2):179–192. doi:10.1093/ejo/23.2.179
8. de Wijer A, de Leeuw JR, Steenks MH, Bosman F. Temporomandibular and cervical spine disorders. Self-reported signs and symptoms. *Spine (Phila Pa 1976)*. 1996;21(14):1638–1646. doi:10.1097/00007632-199607150-00005
9. Gremillion HA. The prevalence and etiology of temporomandibular disorders and orofacial pain. *Tex Dent J*. 2000;117(7):30–39. PMID:11858062.
10. Kitsoulis P, Marini A, Iliou K, et al. Signs and symptoms of temporomandibular joint disorders related to the degree of mouth opening and hearing loss. *BMC Ear Nose Throat Disord*. 2011;11:5. doi:10.1186/1472-6815-11-5
11. Rocabado M. Biomechanical relationship of the cranial, cervical, and hyoid regions. *J Craniomandibular Pract*. 1983;1(3):61–66. doi:10.1080/07345410.1983.11677834
12. Bendtsen L. Central sensitization in tension-type headache – Possible pathophysiological mechanisms. *Cephalalgia*. 2000;20(5):486–508. doi:10.1046/j.1468-2982.2000.00070.x
13. Fink M, Tschernitschek H, Stiesch-Scholz M. Asymptomatic cervical spine dysfunction (CSD) in patients with internal derangement of the temporomandibular joint. *Cranio*. 2002;20(3):192–197. doi:10.1080/08869634.2002.11746210
14. Visscher CM, Lobbezoo F, de Boer W, van der Zaag J, Naeije M. Prevalence of cervical spinal pain in craniomandibular pain patients. *Eur J Oral Sci*. 2001;109(2):76–80. doi:10.1034/j.1600-0722.2001.00996.x
15. Armijo-Olivo S, Fuentes JP, da Costa BR, et al. Reduced endurance of the cervical flexor muscles in patients with concurrent temporomandibular disorders and neck disability. *Man Ther*. 2010;15(6):586–592. doi:10.1016/j.math.2010.07.001
16. Armijo-Olivo S, Silvestre R, Fuentes J, et al. Electromyographic activity of the cervical flexor muscles in patients with temporomandibular disorders while performing the craniocervical flexion test: A cross-sectional study. *Phys Ther*. 2011;91(8):1184–1197. doi:10.2522/ptj.20100233
17. Armijo-Olivo SL, Fuentes JP, Major PW, Warren S, Thie NM, Magee DJ. Is maximal strength of the cervical flexor muscles reduced in patients with temporomandibular disorders? *Arch Phys Med Rehabil*. 2010;91(8):1236–1242. doi:10.1016/j.apmr.2010.05.003
18. Fukunaga T, Miyatani M, Tachi M, Kouzaki M, Kawakami Y, Kanehisa H. Muscle volume is a major determinant of joint torque in humans. *Acta Physiol Scand*. 2001;172(4):249–255. doi:10.1046/j.1365-201x.2001.00867.x
19. De Pauw R, Coppieters I, Kregel J, De Meulemeester K, Danneels L, Cagnie B. Does muscle morphology change in chronic neck pain patients? – A systematic review. *Man Ther*. 2016;22:42–49. doi:10.1016/j.math.2015.11.006
20. Alpayci M, Şenköy E, Delen V, et al. Decreased neck muscle strength in patients with the loss of cervical lordosis. *Clin Biomech (Bristol, Avon)*. 2016;33:98–102. doi:10.1016/j.clinbiomech.2016.02.014
21. Yoon SY, Moon HI, Lee SC, Eun NL, Kim YW. Association between cervical lordotic curvature and cervical muscle cross-sectional area in patients with loss of cervical lordosis. *Clin Anat*. 2018;31(5):710–715. doi:10.1002/ca.23074
22. Ishida H, Suehiro T, Kurozumi C, Ono K, Ando S, Watanabe S. Correlation between neck slope angle and deep cervical flexor muscle thickness in healthy participants. *J Bodyw Mov Ther*. 2015;19(4):717–721. doi:10.1016/j.jbmt.2015.04.001
23. Abbott R, Pedler A, Sterling M, et al. The geography of fatty infiltrates within the cervical multifidus and semispinalis cervicis in individuals with chronic whiplash-associated disorders. *J Orthop Sports Phys Ther*. 2015;45(4):281–288. doi:10.2519/jospt.2015.5719
24. Kaynak BA, Taş S, Salkın Y. The accuracy and reliability of the Turkish version of the Fonseca Anamnestic Index in temporomandibular disorders. *Cranio*. 2023:41(1):78–83. doi:10.1080/08869634.2020.1812808
25. Bevilaqua-Grossi D, Chaves TC, de Oliveira AS, Monteiro-Pedro V. Anamnestic index severity and signs and symptoms of TMD. *Cranio*. 2006;24(2):112–118. doi:10.1179/crn.2006.018
26. da Fonseca DM, Bonfante G, Valle AL, Freitas SFT. Diagnosis of the craniomandibular disfunction through anamnesis [in Portuguese]. *RGO (Porto Alegre)*. 1994;42(1):23–28. https://pesquisa.bvsalud.org/portal/resource/pt/biblio-854978. Accessed March 13, 2023.
27. Young IA, Dunning J, Butts R, Cleland JA, Fernández-de-Las-Peñas C. Psychometric properties of the Numeric Pain Rating Scale and Neck Disability Index in patients with cervicogenic headache. *Cephalalgia*. 2019;39(1):44–51. doi:10.1177/0333102418772584
28. Young IA, Dunning J, Butts R, Mourad F, Cleland JA. Reliability, construct validity, and responsiveness of the neck disability index and numeric pain rating scale in patients with mechanical neck pain without upper extremity symptoms. *Physiother Theory Pract*. 2019;35(12):1328–1335. doi:10.1080/09593985.2018.1471763
29. Ohrbach R, Larsson P, List T. The jaw functional limitation scale: Development, reliability, and validity of 8-item and 20-item versions. *J Orofac Pain*. 2008;22(3):219–230. PMID:18780535.
30. Aslan E, Karaduman A, Yakut Y, Aras B, Simsek IE, Yaglý N. The cultural adaptation, reliability and validity of neck disability index in patients with neck pain: A Turkish version study. *Spine (Phila Pa 1976)*. 2008;33(11):E362–E365. doi:10.1097/BRS.0b013e31817144e1
31. Müller M, Dohrn MF, Romanzetti S, et al. Semi-automated volumetry of MRI serves as a biomarker in neuromuscular patients. *Muscle Nerve*. 2020;61(5):600–607. doi:10.1002/mus.26827
32. Lopes SL, Costa AL, Gamba Tde O, Flores IL, Cruz AD, Min LL. Lateral pterygoid muscle volume and migraine in patients with temporomandibular disorders. *Imaging Sci Dent*. 2015;45(1):1–5. doi:10.5624/isd.2015.45.1.1
33. Liu B, Xu J, Jin Y, et al. Advantages of 3-dimensional measurements for supraspinatus intramuscular fatty evaluation in patients with medium to massive rotator cuff tears: Comparison with a single sagittal slice. *Am J Sports Med*. 2022;50(3):699–707. doi:10.1177/03635465211068854
34. Chung SW, Oh KS, Moon SG, et al. Serial changes in 3-dimensional supraspinatus muscle volume after rotator cuff repair. *Am J Sports Med*. 2017;45(10):2345–2354. doi:10.1177/0363546517706699
35. Valera-Calero JA, Al-Buqain-Ortega A, Arias-Buría JL, Fernández-de-Las-Peñas C, Varol U, Ortega-Santiago R. Echo-intensity, fatty infiltration, and morphology ultrasound imaging assessment in healthy and whiplash associated disorders populations: An observational study. *Eur Spine J*. 2021;30(10):3059–3067. doi:10.1007/s00586-021-06915-z
36. Elliott J, Jull G, Noteboom JT, Darnell R, Galloway G, Gibbon WW. Fatty infiltration in the cervical extensor muscles in persistent whiplash-associated disorders: A magnetic resonance imaging analysis. *Spine (Phila Pa 1976)*. 2006;31(22):E847–E855. doi:10.1097/01.brs.0000240841.07050.34

37. Elliott JM, O'Leary S, Sterling M, Hendrikz J, Pedler A, Jull G. Magnetic resonance imaging findings of fatty infiltrate in the cervical flexors in chronic whiplash. *Spine (Phila Pa 1976)*. 2010;35(9):948–954. doi:10.1097/BRS.0b013e3181bb0e55
38. Naghdi N, Elliott JM, Weber MH, Fehlings MG, Fortin M. Morphological changes of deep extensor neck muscles in relation to the maximum level of cord compression and canal compromise in patients with degenerative cervical myelopathy. *Global Spine J*. 2022:14(4):1184–1192. doi:10.1177/21925682221136492
39. Hallgren RC, Greenman PE, Rechtien JJ. Atrophy of suboccipital muscles in patients with chronic pain: A pilot study. *J Am Osteopath Assoc*. 1994;94(12):1032–1038. PMID:7852102.
40. Uthaikhup S, Assapun J, Kothan S, Watcharasaksilp K, Elliott JM. Structural changes of the cervical muscles in elder women with cervicogenic headache. *Musculoskelet Sci Pract*. 2017;29:1–6. doi:10.1016/j.msksp.2017.02.002
41. Elliott JM, Pedler AR, Jull GA, Van Wyk L, Galloway GG, O'Leary SP. Differential changes in muscle composition exist in traumatic and nontraumatic neck pain. *Spine (Phila Pa 1976)*. 2014;39(1):39–47. doi:10.1097/brs.0000000000000033
42. Fernández-de-las-Peñas C, Albert-Sanchís JC, Buil M, Benitez JC, Alburquerque-Sendín F. Cross-sectional area of cervical multifidus muscle in females with chronic bilateral neck pain compared to controls. *J Orthop Sports Phys Ther*. 2008;38(4):175–180. doi:10.2519/jospt.2008.2598
43. Elliott J, Jull G, Noteboom JT, Galloway G. MRI study of the cross-sectional area for the cervical extensor musculature in patients with persistent whiplash associated disorders (WAD). *Man Ther*. 2008;13(3):258–265. doi:10.1016/j.math.2007.01.012
44. Van Looveren E, Cagnie B, Coppieters I, Meeus M, De Pauw R. Changes in muscle morphology in female chronic neck pain patients using magnetic resonance imaging. *Spine (Phila Pa 1976)*. 2021;46(10):638–648. doi:10.1097/brs.0000000000003856
45. MacDermid JC, Walton DM, Avery S, et al. Measurement properties of the neck disability index: A systematic review. *J Orthop Sports Phys Ther*. 2009;39(5):400–417. doi:10.2519/jospt.2009.2930
46. Alves da Costa DR, de Lima Ferreira AP, Barreto Pereira TA, et al. Neck disability is associated with masticatory myofascial pain and regional muscle sensitivity. *Arch Oral Biol*. 2015;60(5):745–752. doi:10.1016/j.archoralbio.2015.02.009
47. Silveira A, Armijo-Olivo S, Gadotti IC, Magee D. Masticatory and cervical muscle tenderness and pain sensitivity in a remote area in subjects with a temporomandibular disorder and neck disability. *J Oral Facial Pain Headache*. 2014;28(2):138–146. doi:10.11607/ofph.1112
48. Gil-Martínez A, Grande-Alonso M, López-de-Uralde-Villanueva I, López-López A, Fernández-Carnero J, La Touche R. Chronic temporomandibular disorders: Disability, pain intensity and fear of movement. *J Headache Pain*. 2016;17(1):103. doi:10.1186/s10194-016-0690-1
49. Bragatto MM, Bevilaqua-Grossi D, Regalo SCH, Sousa JD, Chaves TC. Associations among temporomandibular disorders, chronic neck pain and neck pain disability in computer office workers: A pilot study. *J Oral Rehabil*. 2016;43(5):321–332. doi:10.1111/joor.12377
50. Coskun Benlidayi I, Guzel R, Tatli U, Salimov F, Keceli O. The relationship between neck pain and cervical alignment in patients with temporomandibular disorders. *Cranio*. 2020;38(3):174–179. doi:10.1080/08869634.2018.1498181
51. Silveira A, Gadotti IC, Armijo-Olivo S, Biasotto-Gonzalez DA, Magee D. Jaw dysfunction is associated with neck disability and muscle tenderness in subjects with and without chronic temporomandibular disorders. *Biomed Res Int*. 2015;2015:512792. doi:10.1155/2015/512792
52. de Abreu Figueirêdo IN, das Graças de Araújo M, Barbosa Fonseca J, et al. Occurrence and severity of neck disability in individuals with different types of temporomandibular disorder. *Oral Maxillofac Surg*. 2021;25(4):471–476. doi:10.1007/s10006-021-00943-1
53. Thorp JN, Willson J. The neck disability index is not correlated with some parameters of temporomandibular disorders: A cross-sectional study. *J Oral Facial Pain Headache*. 2019;33(1):39–46. doi:10.11607/ofph.1992

Original paper

# Evaluation of bite force after microplate and miniplate osteosynthesis for the management of undisplaced or minimally displaced anterior mandibular fractures: A clinical comparative study

Shrikant Shamrao Patil[A–F], Kumar Nilesh[A–F], Mounesh Kumar Chapi[B,C], Prashant Ashok Punde[C,D], Pankaj Patil[C,D], Payal Mate[C,D]

School of Dental Sciences, Krishna Vishwa Vidyapeeth (Deemed to be University), Malkapur, 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*. 2024;61(4):533–539

**Address for correspondence**
Kumar Nilesh
E-mail: drkumarnilesh@yahoo.com

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on May 26, 2021
Reviewed on June 29, 2021
Accepted on July 2, 2021

Published online on August 9, 2024

## Abstract

**Background.** Although the microplate system is commonly used for the treatment of maxillofacial fractures, its use in the fixation of mandibular fractures is not widely accepted.

**Objectives.** The study aimed to evaluate and compare the efficacy of microplates and miniplates in osteosynthesis for the internal fixation of undisplaced and minimally displaced anterior mandibular fractures.

**Material and methods.** A total of 40 patients diagnosed with undisplaced or minimally displaced symphyseal and parasymphyseal fractures were randomly assigned to 2 study groups (group A and group B). Patients in group A (microplate group) were treated with two 0.8-mm microplates, whereas patients in group B (miniplate group) received two 2.0-mm miniplates. Bite force values were recorded in 30 healthy individuals (control group) to establish baseline values. Postoperative bite force values were recorded at various intervals and compared between the study groups and the control group.

**Results.** Both groups demonstrated a progressive improvement in the bite force. However, the bite force values recorded at the 2$^{nd}$, 4$^{th}$ and 6$^{th}$ postoperative weeks were comparatively lower in the microplate group. At the six-week follow-up, the bite force values were lower in both study groups in comparison to the control group. There were no differences in the incidence of postoperative complications between the study groups.

**Conclusions.** The use of microplates in the management of undisplaced or minimally displaced anterior mandibular fractures results in a reduction in the recovery of biting force in comparison to the conventional miniplate system.

**Keywords:** traumatology, maxillofacial, trauma, fracture fixation

**Cite as**
Patil SS, Nilesh K, Chapi MK, Punde PA, Patil P, Mate P. Evaluation of bite force after microplate and miniplate osteosynthesis for the management of undisplaced or minimally displaced anterior mandibular fractures: A clinical comparative study. *Dent Med Probl.* 2024;61(4):533–539. doi:10.17219/dmp/139736

**DOI**
10.17219/dmp/139736

## Introduction

The etiology of facial bone fractures can be attributed to a number of factors, including road traffic accidents, interpersonal violence, falls, and sport-related injuries.[1] Maxillofacial and mandibular injuries can cause facial disfigurement and functional impairment.[2] The fundamental principles of treating mandibular fractures include proper anatomical reduction, restoration of the premorbid occlusion, and appropriate fixation until stable osseointegration. Open reduction and internal fixation are the main treatment modalities for managing mandibular fractures. Semi-rigid fixation techniques include methods such as intraosseous wiring, which allows intersegmental movement across the fracture line. However, this often results in delayed union, malunion, or non-union. Rigid fixation helps overcome these drawbacks by preventing intersegmental movement under active load and includes the use of reconstruction plates, miniplates, lag screws, and compression plates.

Luhr developed the microplate fixation system, revolutionizing the operative management of maxillofacial fractures.[3] The system was designed to reduce the size of bone plates and limit the hardware, with the intention of encouraging close adjustment at the fracture site and minimizing periosteal stripping. In comparison to miniplates, microplates are thinner and more commonly used in the fixation of midface fractures. Microplates have been considered in the treatment of mandibular fractures because their use is less traumatic to soft tissue, requires less periosteal tissue reflection, minimum tissue interference, and easier contouring that provides three-dimensional (3D) geometric stability. Furthermore, reduced palpation and thermal conduction due to the thin cutaneous cover improve patient comfort.[4]

## Objectives

The present prospective clinical study aimed to evaluate the efficacy of microplates for managing undisplaced or minimally displaced anterior mandibular fractures, and to compare them with miniplate osteosynthesis in terms of occlusal stability, fracture fixation and biting efficiency using a bite force measurement device.

## Material and methods

This prospective randomized clinical study was conducted in patients aged 18–35 years who required open reduction and internal fixation for undisplaced or minimally displaced symphyseal or parasymphyseal fractures. A total of 70 patients were enrolled in the study, including 30 healthy individuals as a control group and a study group of 40 patients with undisplaced or minimally displaced anterior mandibular fractures. The study was approved by the Krishna Institute of Medical Sciences Ethics Committee (approval No. KIMSDU/IEC/02/2018). Patients who expressed willingness to participate and who provided informed consent were included in the study. The patients with mandibular fractures (study group) were randomly divided into group A, treated with 2 microplates (0.8-mm thick) and 8 microscrews (1.5 mm × 8 mm and 1.5 mm × 10 mm), and group B, treated with 2 miniplates (2.0 mm) and 8 miniscrews (2 mm × 8 mm and 2 mm × 10 mm), according to an odd-even formula. Each study group consisted of 20 patients. Thirty healthy individuals whose age and gender matched the study group were recruited for the control group.

Patients with comminuted mandibular fractures, concomitant midface and dentoalveolar fractures, infected fractures, partial or complete edentulism, and systemic diseases were excluded from the study. Detailed preoperative medical and clinical examinations were conducted. The diagnosis of undisplaced or minimally displaced symphyseal or parasymphyseal fractures was based on clinical and radiographic assessments. Orthopantomograms were used to evaluate mandibular lower border displacement, with displacements of less than 5 mm included in the study (Fig. 1A). Advanced diagnostic techniques, including low-dose radiation and 3D assessments using computed tomography (CT), cone beam computed tomography and magnetic resonance imaging, facilitate comprehensive evaluation of head and neck disorders, including the assessment of both soft and hard tissue injuries.[5–7] Therefore, CT with 3D reconstruction of the face was performed, with fractures exhibiting bucco-lingual overlap of less than 5 mm included in the study (Fig. 1B).

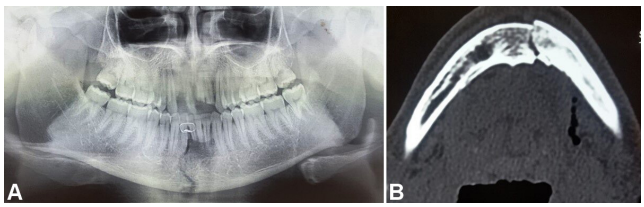


Fig. 1. Preoperative radiographic evaluation of the anterior mandible fracture
A. Displacement of <5 mm of the lower border on the mandible on the orthopantomograph (OPG); B. Displacement of <5 mm of the buccal cortex on the axial computed tomography (CT) scan.

### Surgical procedure

Following the pre-anesthetic check-up, each patient underwent surgery under general anesthesia with nasal intubation. The standard surgical preparation and draping were performed under strict aseptic conditions.

Local anesthesia (2% lidocaine with 1:200,000 adrenaline) was injected at the fracture site. Maxillary and mandibular arch bars were placed for intermaxillary fixation. An intraoral mandibular incision was made according to the location of the fracture, and a full-thickness mucoperiosteal flap was elevated, retracting the tissues. Cautery was occasionally used during dissection to maintain a bloodless field. The fracture segments were adequately exposed, followed by anatomical reduction and intermaxillary fixation to achieve passive occlusion.

In group A, fixation was performed using 2 titanium microplates (0.8-mm, 4-hole design with a gap) and 8 microscrews (1.5 mm × 8 mm and 1.5 mm × 10 mm), which were placed subapically and at the inferior border of the mandible according to Champy's lines of osteosynthesis (Fig. 2–4).

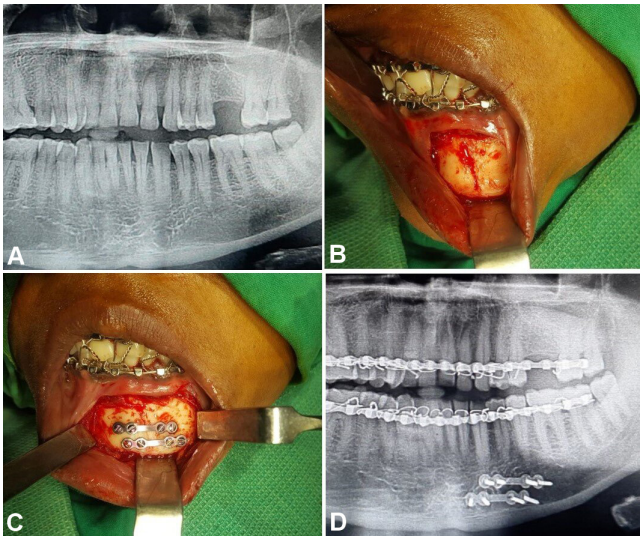


**Fig. 2.** Operative procedure for microplate fixation (group A, case 1)

A. Preoperative OPG; B. Exposure of the fractured segment; C. Fixation using microplates; D. Postoperative OPG.

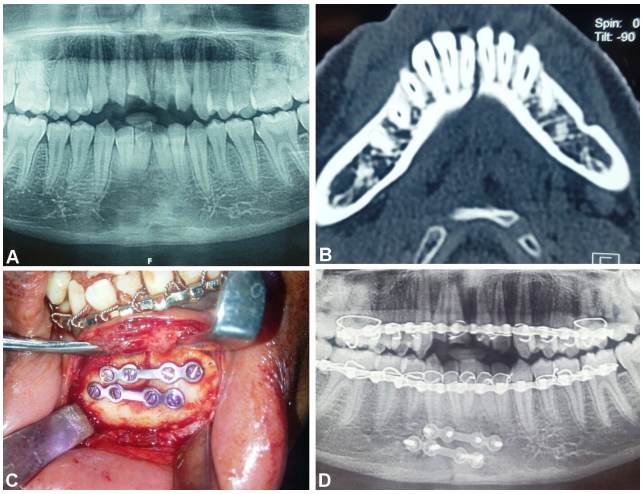


**Fig. 3.** Operative procedure for microplate fixation (group A, case 9)

A. Preoperative OPG; B. Preoperative axial section of the CT scan; C. Fixation using microplates; D. Postoperative OPG.

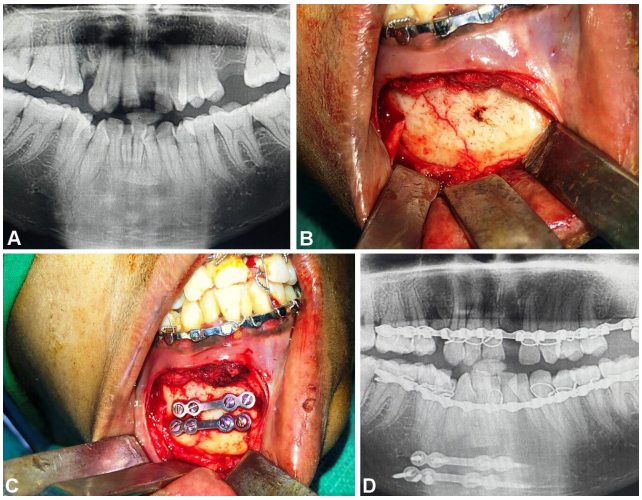


**Fig. 4.** Operative procedure for microplate fixation (group A, case 13)

A. Preoperative OPG; B. Intraoperative exposure of the fracture site; C. Fixation using microplates; D. Postoperative OPG.

In group B, fixation was conducted using 2 titanium miniplates (2.0-mm, 4-hole design with a gap) and 8 miniscrews (2 mm × 8 mm and 2 mm × 10 mm), which were positioned subapically and at the inferior border of the mandible according to Champy's lines of osteosynthesis (Fig. 5–7).

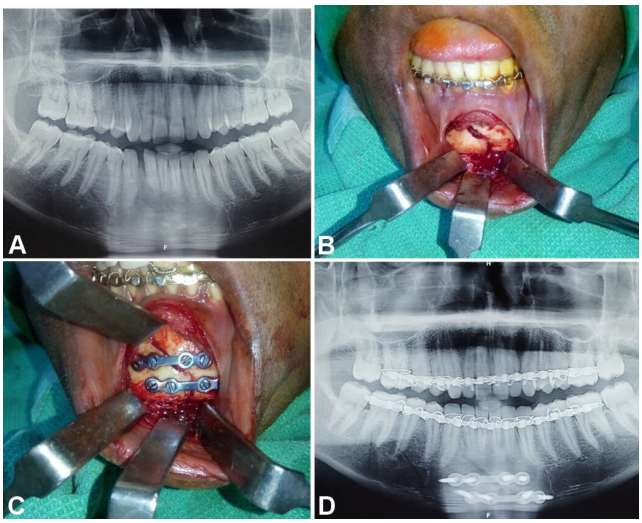


**Fig. 5.** Operative procedure for miniplate fixation (group B, case 7)

A. Preoperative OPG; B. Exposure of the fractured segment; C. Fixation using miniplates; D. Postoperative OPG.

Following the fixation procedure, the intermaxillary fixation was removed, and the occlusion was checked passively. The wound was closed using 3-0 absorbable Vicryl sutures in a continuous locking pattern for all patients. In accordance with the established trauma protocol, perioperative antibiotics and analgesics were administered. The surgical technique was identical for both groups and was performed by the same operating surgeon. All patients received postoperative antibiotic coverage for 5 days and were advised to

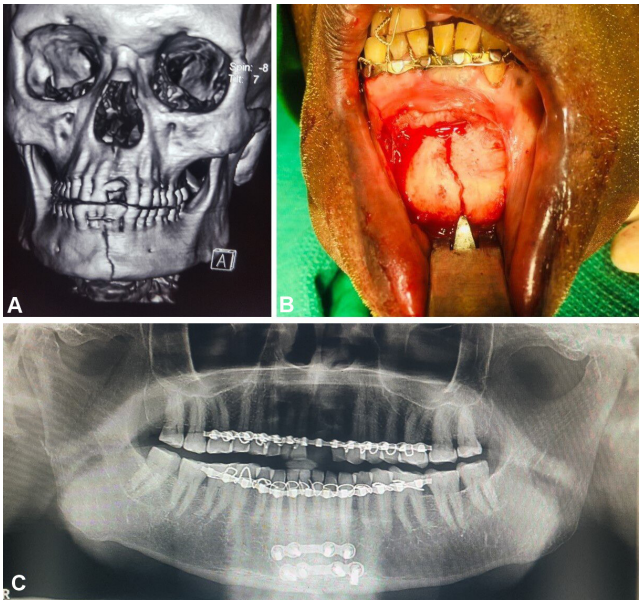


Fig. 6. Operative procedure for miniplate fixation (group B, case 14)
A. Preoperative three-dimensional (3D) CT; B. Exposure of the fractured segment; C. Postoperative OPG showing miniplate fixation.

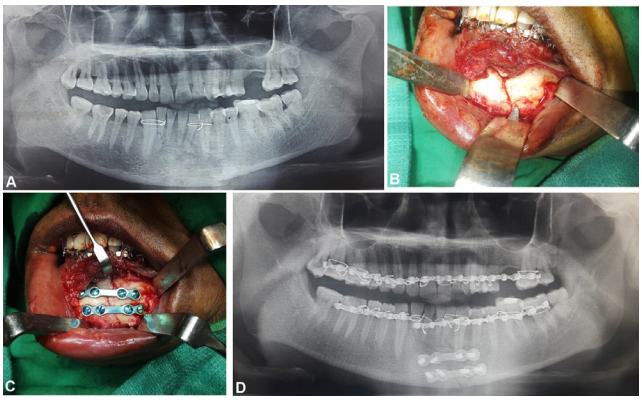


Fig. 7. Operative procedure for miniplate fixation (group B, case 17)
A. Preoperative OPG; B. Exposure of the fractured segment; C. Fixation using miniplates; D. Postoperative OPG.

consume a liquid and semisolid diet for 2 to 3 weeks. The subjects were instructed to rinse frequently with chlorhexidine mouthwash. Patients were scheduled for follow-up assessments at postoperative weeks 1, 2, 4, and 6. The parameters assessed included the bite force, occlusal discrepancy, segment mobility, plate integrity, and fracture site infection.

Bite force measurements were recorded using a digital bite force recorder (HARIOM Electronics, Vadodara, India). In the study groups, the bite force was measured at the incisors, right molars and left molars before surgery and at 1, 2, 4, and 6 weeks after surgery. A single bite force measurement was recorded for the control group at the incisors, right molars and left molars in a similar manner. The data was tabulated and analyzed statistically using unpaired *t*-tests and analysis of variance (ANOVA) with Tukey's post hoc tests.

## Results

A total of 70 patients were enrolled in the study, comprising 30 healthy individuals (control group) and 40 patients with undisplaced or minimally displaced anterior mandibular fractures (groups A and B). In the control group, 16 individuals were male and 14 were female. In study group A, 14 of the 20 patients were male and 6 were female, whereas in group B, 16 of the 20 patients were male and 4 were female. There was no statistically significant difference observed in sex distribution between the study and control groups ($p = 0.684$).

The age of the healthy individuals in the control group ranged from 19 to 35 years, with a mean of 27.17 years. In group A, the mean age was 27.50 years (range: 18–35 years), and in group B, it was 27.55 years (range: 19–35 years). No statistically significant difference was observed in age distribution among the 3 groups ($p = 0.134$).

A comparison of the bite force between study groups A and B was performed using unpaired *t*-tests at various time intervals (Fig. 8,9). Preoperatively, in group A,

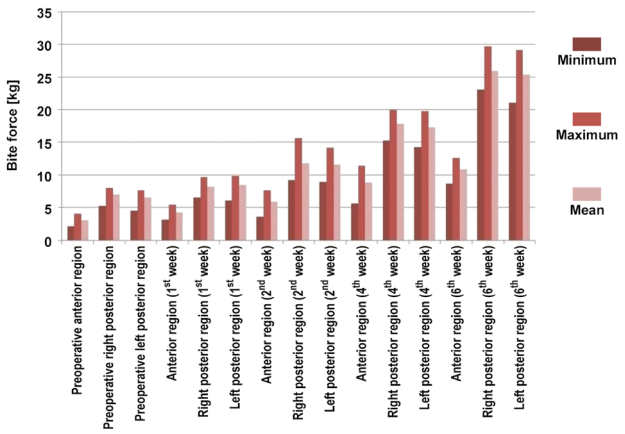


Fig. 8. Descriptive statistics for the bite force in group A at the anterior, right posterior and left posterior regions

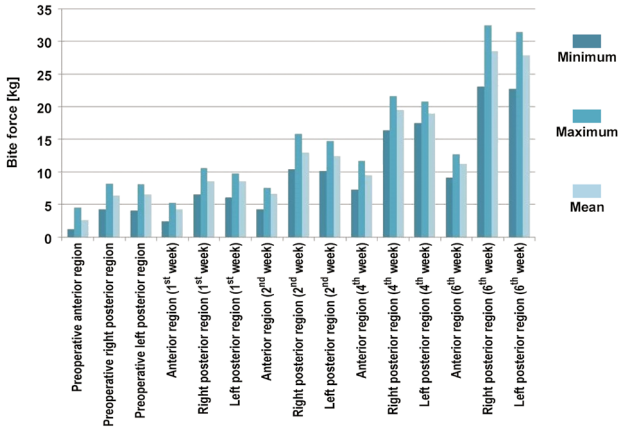


Fig. 9. Descriptive statistics for the bite force in group B at the anterior, right posterior and left posterior regions

the mean bite forces at the anterior, right posterior and left posterior regions were 3.08 kg, 6.97 kg and 6.58 kg, respectively. In group B, the mean bite forces at the anterior, right posterior and left posterior regions were 2.55 kg, 6.35 kg and 6.50 kg, respectively. A statistically significant difference was identified in the preoperative comparison of the anterior bite force between group A and group B ($p$ = 0.014), with a mean difference of 0.534 kg. The mean differences at the right posterior (0.621 kg) and left posterior (0.065 kg) regions were found to be insignificant ($p$ = 0.051 and $p$ = 0.822, respectively).

After the 1st postoperative week, the mean bite force at the anterior region for group A was 4.22 kg, while the mean bite force at the right and left posterior regions was 8.18 kg and 8.50 kg, respectively. In contrast, in group B, the mean bite force at the anterior region was 4.20 kg, and at the right and left posterior regions it was 8.56 kg and 8.56 kg, respectively. The mean differences between group A and group B for the anterior, right posterior and left posterior regions were 0.017 kg, –0.378 kg and –0.665 kg, respectively. These differences were found to be statistically insignificant ($p$ = 0.943, $p$ = 0.257 and $p$ = 0.835).

After the 2nd postoperative week, the mean bite force in group A was 5.90 kg at the anterior region, 11.70 kg at the right posterior region and 11.60 kg at the left posterior region. In contrast, in group B, it was 6.61 kg at the anterior region, 13.02 kg at the right posterior region and 12.40 kg at the left posterior region. The mean difference between group A and group B for the right posterior region was –1.264 kg, which was statistically significant ($p$ = 0.021). However, the mean differences between group A and group B for the anterior (–0.709 kg) and left posterior regions (–0.823 kg) were insignificant ($p$ = 0.059 and $p$ = 0.080, respectively).

After the 4th postoperative week, the mean bite forces in group A at the anterior, right posterior and left posterior regions were 8.82 kg, 17.80 kg and 17.30 kg, respectively. In contrast, in group B, the mean bite force values at the anterior, right posterior and left posterior regions were 9.45 kg, 19.46 kg and 18.90 kg, respectively. The mean difference between group A and group B at the right and left posterior regions was –1.620 kg and –1.595 kg, respectively, which was found to be statistically significant ($p$ = 0.001 and $p \leq 0.001$, respectively). However, at the anterior region, the mean difference was statistically insignificant (–0.632 kg; $p$ = 0.134).

Lastly, after the 6th postoperative week, in group A, the mean bite forces were 10.80 kg, 25.90 kg and 25.30 kg at the anterior, right posterior and left posterior regions, respectively. In group B, the corresponding values were 11.23 kg, 28.52 kg and 27.86 kg at the anterior, right posterior and left posterior regions, respectively. The mean difference between group A and group B at the right posterior and left posterior regions was –2.560 kg and –2.530 kg, respectively, which was found to be significant ($p$ = 0.001 for both). However, at the anterior region, the mean difference was not statistically significant ($p$ = 0.359), with a value of –0.350 kg.

A comparison of the bite force among the control group and study groups (A and B) at the 6th postoperative week was conducted using the ANOVA Tukey's post hoc test. The mean difference in the bite force at the anterior region between the control group and the study groups was 5.703 kg ($p \leq 0.001$) and 5.353 kg ($p \leq 0.001$), respectively, which was statistically significant. Similarly, the mean difference in the bite force at the right posterior region between the control group and the study groups was 18.648 kg ($p \leq 0.001$) and 16.088 kg ($p \leq 0.001$), respectively. At the left posterior region, the mean difference in the bite force was 17.223 kg ($p < 0.001$) and 14.693 kg ($p \leq 0.001$), respectively. The observed difference in the bite force between the study and control groups in both the right and left molar regions was statistically significant (Table 1).

All patients in the study groups were followed up for 1 year. They were evaluated for the presence of complications, including occlusal discrepancy, mobility between segments, infection at the fracture site, and plate fracture. Two patients in group A and 2 patients in group B experienced fracture site infection at the 12th and 13th weeks and at the 11th and 16th weeks, respectively. This was effectively managed with antibiotics, and no further intervention was required. There were no complications observed in either of the study groups.

**Table 1.** Comparison of the bite force between the control group and the study groups at the 6th postoperative week

| Location | Control group (C) | Study group (S) | Mean difference (C–S) | *SE* | *p*-value | 95% *CI* | |
|---|---|---|---|---|---|---|---|
| | | | | | | lower bound | upper bound |
| Anterior region | control group | group A | 5.702 | 0.731 | <0.001* | 3.951 | 7.455 |
| | | group B | 5.353 | 0.731 | <0.001* | 3.601 | 7.105 |
| Right posterior region | control group | group A | 18.648 | 2.002 | <0.001* | 13.849 | 23.448 |
| | | group B | 16.088 | 2.002 | <0.001* | 11.289 | 20.888 |
| Left posterior region | control group | group A | 17.223 | 1.693 | <0.001* | 13.166 | 21.281 |
| | | group B | 14.693 | 1.693 | <0.001* | 10.636 | 18.751 |

* statistically significant ($p < 0.05$, Tukey's post hoc test); *SE* – standard error; *CI* – confidence interval.

## Discussion

Arbeitsgemeinschaft Osteosynthesefragen/Association for the Study of Internal Fixation (AO/ASIF) recommendations for rigid fixation are based on 4 fundamental principles: bony segment reduction; stable fixation; immobilization of fragments to maintain adequate blood supply; and early return of function.[8] Advancements in techniques, biomaterials, and understanding of biophysics have significantly influenced the application of fixation in facial fractures. Internal fixation with titanium hardware is the most commonly used method for the treatment of facial skeletal injuries, with numerous systems available for this purpose.[9]

A miniplate is a semi-rigid fixation system designed to achieve multiple points of bone fragment fixation, thereby maintaining facial dimensions, preventing rotational movement of fragments, and providing interfragment stability. Adverse outcomes associated with miniplates that may necessitate removal include plate prominence, palpability, infection, migration, and exposure.[10] In mandibular fractures, infection and exposure are the most common reasons for plate removal, whereas prominence and pain are primary concerns in midface fractures.[7] A review involving 507 patients (1,112 facial fractures) demonstrated that 12% of patients required miniplate removal due to various reasons.[11] To reduce the risk of postoperative complications and the need for implant removal, it is desirable to minimize the size and amount of material used.

It has been reported that metal deposits can occur in close proximity to titanium miniplates and in peripheral organs after osteosynthesis.[12] Additionally, the implant used should require a minimal degree of periosteal stripping. The preservation of vital periosteum facilitates fracture healing, reduces the likelihood of postoperative wound breakdown, and lowers the incidence of hardware-related infections. Therefore, it is logical to minimize the quantity of osteosynthesis material used.

The use of microplate systems in the craniofacial region was first presented by Hans Luhr in Atlanta in November 1987.[13] Clinical indications for the use of microplate systems include naso-ethmoid, infraorbital, frontal, and calvarial fractures.[14,15] These systems offer greater adaptability to the contours of the midface skeleton due to their easier malleability. Their lower profile reduces plate palpability and pain, while also lowering the risk of exposure during midface fracture treatment.[16–18]

The use of microplates in the treatment of mandibular fractures is limited, with only a few papers reporting on its efficacy.[12,19–24] Recent experimental and clinical studies have shown favorable outcomes with the use of microplates in the stress-bearing areas of the mandible. An experimental study demonstrated that microplates have reduced load-bearing ability in comparison to miniplates. However, microplates possess nearly equal torsional force and sufficient stability for mandibular osteosynthesis. In a prospective study, Song et al. concluded that mandibular fractures can be effectively fixed with 2- or 3-point fixation using microplates, without the need for maxillomandibular fixation, ensuring good stability, adaptation and patient comfort.[21] Similar results were published by Kumar et al. on the treatment of inter-foraminal mandibular fractures.[22] The torsional strength of microplates was found to be similar to that of the miniplate system.

The primary goal of fracture management is to facilitate the early return of function. The early mobilization of the jaw and the restoration of adequate bite force for mastication should be the primary objectives in managing mandibular fractures.[15] In the present study, the bite force was evaluated as the primary treatment outcome. Similar use of the bite force for evaluating the efficacy of microplates in the fixation of mandible fractures has been reported in the literature.[12,20,22]

The present study demonstrated a progressive improvement in the bite force in both study groups. However, the bite force measurements at all intervals were consistently lower in the microplate group compared to the miniplate group. This difference was statistically significant at the 2$^{nd}$, 4$^{th}$ and 6$^{th}$ postoperative weeks. This finding is in agreement with the results of a previously published study by Ahmed et al.[20] Contrary to our findings, Anand et al.[19] reported no significant difference in bite force values between the 2 plating systems. However, their study design differed, with 2 miniplates in 1 group and a combination of 1 miniplate and 1 microplate in the other.

In comparison to the control group, patients in both study groups showed a reduction in the bite force at the last follow-up (6$^{th}$ week). This suggests that the bite force of patients treated with either microplates or miniplates does not return to its baseline values by the 6$^{th}$ week of fracture healing. Two patients from each study group experienced surgical site infections, which were managed conservatively with antibiotics. There were no reports of fracture segment mobility, plate/screw loosening, or occlusal discrepancy in either group.

Although this study used microplate fixation in adult cases of undisplaced or minimally displaced anterior mandibular fractures, its use in the pediatric population and for comminuted mandibular fractures has also been documented. The low profile and malleability of microplates facilitate better adaptation and minimize trauma to tooth buds in young patients.[23] Choi et al. found that microplates are strong enough to maintain a reduction in comminuted mandibular fractures.[24] Their small size and malleability allow for multiple fixations of comminuted bony segments in an accurate anatomical position with less periosteal stripping and self-occlusal adjustment. However, in the present study, the degree of periosteal stripping required for hardware fixation was not found to be similar in both study groups.

## Conclusions

The application of 2 microplates resulted in a reduction in bite force values at the six-week follow-up compared to the miniplate and control groups. Based on the findings of this study, the use of microplates in the management of undisplaced or minimally displaced anterior mandibular fractures should be discouraged. However, further prospective randomized controlled studies with a large sample size and longer follow-up are essential to evaluate the suitability of this system for regular use.

## Ethics approval and consent to participate

The study was approved by the Krishna Institute of Medical Sciences Ethics Committee (approval No. KIMSDU/IEC/02/2018).

## 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

Shrikant Shamrao Patil https://orcid.org/0000-0002-3231-7526
Kumar Nilesh https://orcid.org/0000-0001-7771-5475
Mounesh Kumar Chapi https://orcid.org/0000-0002-6763-4708
Prashant Ashok Punde https://orcid.org/0000-0003-4880-5644
Pankaj Patil https://orcid.org/0000-0001-5889-8595
Payal Mate https://orcid.org/0000-0002-3369-3312

### References

1. Singaram M, Vijayabala S, Udhayakumar RK. Prevalence, pattern, etiology, and management of maxillofacial trauma in a developing country: A retrospective study. *J Korean Assoc Oral Maxillofac Surg*. 2016;42(4):174–181. doi:10.5125/jkaoms.2016.42.4.174
2. Parashar A, Sharma RK. Unfavorable outcomes in maxillofacial injuries: How to avoid and manage. *Indian J Plast Surg*. 2013;46(2):221–234. doi:10.4103/0970-0358.118597
3. Luhr HG. Indications for use of a microsystem for internal fixation in craniofacial surgery. *J Craniofac Surg*. 1990;1(1):35–52. doi:10.1097/00001665-199001000-00009
4. Sadhwani BS, Anchlia S. Conventional 2.0 mm miniplates versus 3-D plates in mandibular fractures. *Ann Maxillofac Surg*. 2013;3(2):154–159. doi:10.4103/2231-0746.119231
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. 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;4(2):36. doi:10.3390/mps4020036
7. Reda R, Zanza A, Mazzoni A, Cicconetti A, Testarelli L, Di Nardo D. An update of the possible applications of magnetic resonance imaging (MRI) in dentistry: A literature review. *J Imaging*. 2021;7(5):75. doi:10.3390/jimaging7050075
8. Brown JS, Grew N, Taylor C, Millar BG. Intermaxillary fixation compared to miniplate osteosynthesis in the management of the fractured mandible: An audit. *Br J Oral Maxillofac Surg*. 1991;29(5):308–311. doi:10.1016/0266-4356(91)90116-m
9. Feller KU, Richter G, Schneider M, Eckelt U. Combination of microplate and miniplate for osteosynthesis of mandibular fractures: An experimental study. *Int J Oral Maxillofac Surg*. 2002;31(1):78–83. doi:10.1054/ijom.2000.0182
10. Campbell CA, Lin KY. Complications of rigid internal fixation. *Craniomaxillofac Trauma Reconstr*. 2009;2(1):41–47. doi:10.1055/s-0029-1202596
11. Francel TJ, Birely BC, Ringelman PR, Manson PN. The fate of plates and screws after facial fracture reconstruction. *Plast Reconstr Surg*. 1992;90(4):568–573. doi:10.1097/00006534-199210000-00004
12. Gupta A, Singh V, Mohammad S. Bite force evaluation of mandibular fractures treated with microplates and miniplates. *J Oral Maxillofac Surg*. 2012;70(8):1903–1908. doi:10.1016/j.joms.2012.03.036
13. Beals SP, Munro IR. The use of miniplates in craniomaxillofacial surgery. *Plast Reconstr Surg*. 1987;79(1):33–38. doi:10.1097/00006534-198701000-00005
14. Farole A, Diecidue RJ. Microscrew and microplate systems for select osteotomies in orthognathic surgery. *Oral Surg Oral Med Oral Pathol*. 1993;75(3):276–279. doi:10.1016/0030-4220(93)90134-p
15. Schortinghuis J, Bos RR, Vissink A. Complications of internal fixation maxillofacial fractures with microplates. *J Oral Maxillofac Surg*. 1999;57(2):130–134; discussion 135. doi:10.1016/s0278-2391(99)90224-0
16. Jack JM, Stewart DH, Rinker BD, Vasconez HZ, Pu LLQ. Modern surgical treatment of complex facial fractures: A 6-year review. *J Craniofac Surg*. 2005;16(4):726–731. doi:10.1097/01.scs.0000168766.33238.22
17. Evans GR, Clark N, Manson PN, Leipziger IS. Role of mini- and microplate fixation in fractures of midface and mandible. *Ann Plast Surg*. 1995;34(5):453–456. doi:10.1097/00000637-199505000-00001
18. Burm JS, Hansen JE. The use of microplates for internal fixation of mandibular fractures. *Plast Reconstr Surg*. 2010;125(5):1485–1492. doi:10.1097/PRS.0b013e3181d51244
19. Anand D, Prasad K, Lalitha RM, et al. Bite force assessment of mandibular interforaminal fractures treated with combination of microplate and miniplate – A randomized control study. *Craniomaxillofac Trauma Reconstr*. 2018;2(1):e1–e8. doi:10.1055/s-0038-1646776
20. Ahmed SS, Bhardwaj S, Ansari MK, Farooq O, Khan AA. Role of 1.5 mm microplates in treatment of symphyseal fracture of mandible: A stress analysis based comparative study. *J Oral Biol Craniofac Res*. 2017;7(2):119–122. doi:10.1016/j.jobcr.2017.03.009
21. Song SW, Burm JS, Yang WY, Kang SY. Microplate fixation without maxillomandibular fixation in double mandibular fractures. *Arch Craniofac Surg*. 2014;15(2):53–58. doi:10.7181/acfs.2014.15.2.53
22. Kumar ST, Saraf S, Devi SP. Evaluation of bite force after open reduction and internal fixation using microplates. *J Dent (Tehran)*. 2013;10(5):466–477. PMID:24910656.
23. Sheta MS, Shoushan MM, Hussein MM, Abd Elaal SE. Evaluation of using microplates osteosynthesis for pediatric mandibular fractures. *Tanta Dent J*. 2015;12(3):149–155. doi:10.1016/j.tdj.2015.01.002
24. Choi TJ, Burm JS, Chung YH. Abstract: A microplate fixation for treatment of comminuted mandibular fractures. *Plast Reconstr Surg Glob Open*. 2017;5(9 Suppl):159–160. doi:10.1097/01.GOX.0000526394.30830.25

Original paper

# Comparative evaluation of retention, marginal adaptation and marginal discoloration of hydrophilic and hydrophobic pit and fissure sealants: A split-mouth randomized controlled trial

Sanjana Ghadge[A,C,D], Farhin Katge[A,E,F], Manohar Poojari[B,C], Khushboo Jain[B,D], Vamsi Krishna Chimata[C–E]

Department of Pediatric and Preventive Dentistry, Terna Dental College, Navi Mumbai, 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*. 2024;61(4):541–548

**Address for correspondence**
Farhin Katge
E-mail: pedotdc@gmail.com

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
The authors would like to thank Aishwarya Kamble and Komal Nanavati for data collection and Deepak Langade, from the Department of Public Health Dentistry (Terna Dental College), for statistical analysis of the study.

Received on March 13, 2022
Reviewed on April 29, 2022
Accepted on May 4, 2022

Published online on August 9, 2024

**Cite as**
Ghadge S, Katge F, Poojari M, Jain K, Krishna Chimata V. Comparative evaluation of retention, marginal adaptation and marginal discoloration of hydrophilic and hydrophobic pit and fissure sealants: A split-mouth randomized controlled trial. *Dent Med Probl.* 2024;61(4):541–548. doi:10.17219/dmp/149732

**DOI**
10.17219/dmp/149732



## Abstract

**Background.** Pit and fissure sealants are the most commonly used preventive measure against caries in permanent molars. Advancements in dental materials have led to the development of hydrophilic sealants. However, their clinical efficacy must be evaluated and compared with that of conventional hydrophobic sealants.

**Objectives.** This study aimed to clinically evaluate and compare the retention, marginal adaptation and marginal discoloration of hydrophilic and hydrophobic pit and fissure sealants over a 12-month follow-up period.

**Material and methods.** The study was a split-mouth, double-blind, randomized controlled trial. A sample size calculation was performed, and 120 first permanent molars (60 in each group) were selected for inclusion in the study. According to the split-mouth design, the sample was randomly divided into 2 groups. Group A was treated with a hydrophilic sealant (UltraSeal® XT Hydro), while Group B was treated with a hydrophobic sealant (Conseal F). The sealants in both groups were applied in accordance with the manufacturer's instructions by a single operator. The sealants were evaluated clinically using visual and tactile methods by 2 independent examiners who were blinded to the procedure in accordance with the modified United States Public Health Service (USPHS) clinical rating system at placement and at 1, 3, 6, and 12 months.

**Results.** After 12 months of follow-up, the Conseal F sealant showed significantly better retention ($p = 0.001$), marginal adaptation ($p = 0.023$) and reduced marginal discoloration ($p = 0.004$) in comparison to the UltraSeal XT Hydro sealant.

**Conclusions.** The Conseal F (hydrophobic) sealant demonstrated superior retention, marginal adaptation and marginal discoloration compared to the UltraSeal XT Hydro (hydrophilic) sealant.

**Keywords:** first permanent molars, Conseal F, resin-based sealant, UltraSeal XT Hydro

# Introduction

The occlusal surfaces of posterior teeth are highly susceptible to caries due to bacterial accumulations being confined within the complex morphology of pits and fissures.[1] The prevalence of caries on the occlusal surfaces of molars in children aged 5–17 years has been reported to range from 67% to 90%.[2,3]

One of the most effective methods for preventing caries in pits and fissures is the application of pit and fissure sealants.[4] These materials are introduced into the occlusal pits and fissures of caries-susceptible teeth, forming a micromechanically bonded protective layer that blocks the access of caries-producing bacteria and their source of nutrients.[5] Pit and fissure sealants offer several advantages, including a lower cost compared to restorations and a nine-fold reduction in caries occurrence compared to unsealed teeth.[6]

Resin-based filled and unfilled fluoridated sealants, incorporating fluoride as a caries-preventive ingredient, have been introduced. Their effectiveness is attributed to their retention because they penetrate the micropores of etched enamel surfaces. Resin-based sealants are considered effective in the prevention of caries due to their higher retention rates and proven cariostatic effects. However, their application is technique-sensitive and depends on the practitioner's skill, the patient's cooperation and the prevention of salivary contamination. Additionally, their hydrophobic nature makes them highly susceptible to moisture contamination.[7]

The recent introduction of hydrophilic sealants has the potential to reduce microleakage and enhance retention.[8,9] UltraSeal® XT Hydro is a moisture-tolerant resin-based sealant that is capable of bonding to slightly moist tooth structures, thereby creating an impervious interface. The oral cavity is a 100% humid environment where moisture control is difficult to achieve. In such conditions, the use of a moisture-tolerant sealant may be advantageous.[10]

The caries-preventive effect of pit and fissure sealants depends on their retention and marginal adaptation. Therefore, this study aimed to clinically evaluate and compare the retention, marginal adaptation and marginal discoloration of UltraSeal XT Hydro and Conseal F pit and fissure sealants.

# Material and methods

The study was conducted in the Department of Pediatric and Preventive Dentistry at Terna Dental College in Navi Mumbai, India. The ethical approval was obtained from the Institutional Review Board (reference No. TDC/IRB-EC/145/2017). The study was registered with the Clinical Trial Registry India under the code CTRI/2018/08/015206. The trial was conducted in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines (Fig. 1).

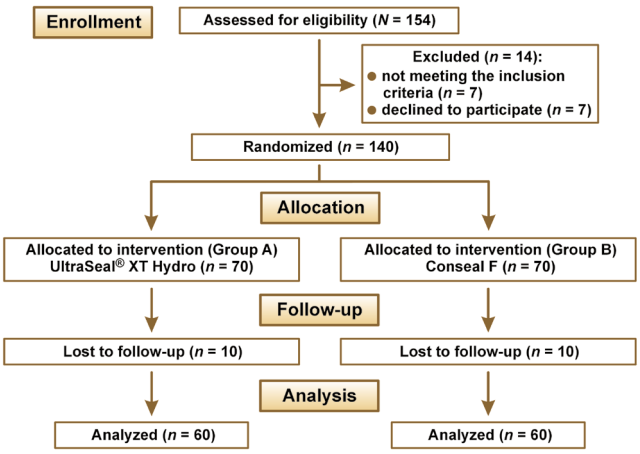


Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flowchart

## Study design and sample size calculation

The study was designed as a split-mouth, double-blind, randomized controlled trial, ensuring that both patients and examiners were unaware of the sealants used. The sample size calculated per group was 60, with an alpha of 0.05 and a beta of less than 0.2 (power >80%). The assumed retention rate was 72% in Group A (hydrophilic sealant) and 50% in Group B (hydrophobic sealant). The proportion in Group A was assumed to be 0.5000 under the null hypothesis and 0.7200 under the alternative hypothesis.[11] To account for potential dropouts, the sample size was increased by 15%, and the study was initiated with 140 sealants.

## Patient selection and recruitment

Prior to the procedure, the parents provided written informed consent. Both children and parents received detailed verbal explanations regarding the procedure. The children were blinded to the type of sealant used.

A total of 64 children were initially assessed for eligibility. Of these, 55 children met the inclusion and exclusion criteria and were thus selected for the study.

### Inclusion criteria

The study included children between the ages of 6 and 9 who exhibited a high risk of caries and Frankl's behavior ratings of 3 or 4. Fully erupted, caries-free maxillary and mandibular first permanent molars with deep retentive pits and fissures were also included in the study. These criteria were evaluated through a clinical examination of the teeth using a mirror and a blunt probe, as well as the analysis of bitewing radiographs.

### Exclusion criteria

Maxillary and mandibular molars with incipient caries, deep cavitated carious lesions, existing restorations, or previous sealants on occlusal surfaces, as well as hypomineralized molars and those with developmental disturbances were excluded from the study. Additionally, the participants included in the study were not using any removable or fixed dental appliances.[11]

## Randomization

The selected teeth were randomly divided into 2 groups (Group A and Group B) using the computer-assisted randomization software (Rando 1.2 v. 2004; JIPMER, Puducherry, India). The allocation concealment was achieved with the use of sequentially labeled opaque envelopes. The allocation was performed by an independent individual. Each group (Group A and Group B) consisted of 70 first permanent molars (35 maxillary and 35 mandibular). In Group A, a hydrophilic sealant (UltraSeal XT Hydro) was applied, while Group B was treated with a hydrophobic sealant (Conseal F).

The sealants were applied in accordance with the manufacturer's instructions by a single operator, who also calibrated the clinical protocol for the procedure.

In Group A, the tooth was isolated from the oral cavity with the use of a rubber dam. Prophylaxis was conducted using a slow-speed contra-angle micromotor handpiece with a bristle brush and polishing paste. The tooth was thoroughly rinsed with water for 30 s, after which it was dried with a three-way syringe. Acid etching was conducted for 20 s using 35% Ultra-Etch phosphoric acid gel (Ultradent Products, Inc., South Jordan, USA). Subsequently, the etched surface was rinsed with water for 30 s and slightly air-dried to remove any standing or pooled water. The tooth surface was treated with care to prevent desiccation. UltraSeal XT Hydro was applied with a brush tip and distributed evenly across all pits and fissures on the tooth surface. The sealant was light-cured for 20 s (Bluephase N MC; Ivoclar Vivadent, New York, USA). Then, the sealant was examined for marginal adaptation with an explorer, and the evaluation of the occlusion was conducted.[12]

In Group B, the tooth was isolated with a rubber dam, and prophylaxis was performed as described above. The tooth was rinsed thoroughly with water for 30 s and dried with a three-way syringe. Acid etching was performed for 20 s using 35% Ultra-Etch phosphoric acid gel (Ultradent Products, Inc.), followed by rinsing with water for 30 s. Then, the tooth was air-dried for 15 s using a three-way syringe, and checked for a frosted appearance. If a frosted appearance was not achieved, additional 10 s of etching were performed. Then, the stae total etch adhesive system (SDI Limited, Melbourne, Australia) was applied. The tooth surface was slightly air-dried to facilitate the even distribution of the bonding agent, which was then light-cured for 20 s. The Conseal F sealant was applied by placing the sealant tip on the mesial pit and spreading it over all pits and fissures of the maxillary molars. The same process was carried out on the mandibular molars, ensuring that no air bubbles were incorporated. The sealant was light-cured for 20 s and examined for marginal adaptation with an explorer.[11]

## Clinical evaluation

Clinical evaluations of the sealants were performed by 2 independent, calibrated examiners who were blinded to both study groups. All sealants were evaluated according to the modified United States Public Health Service (USPHS) clinical rating system at baseline and at 1, 3, 6, and 12 months (Table 1).[13,14] At each recall visit, any plaque and debris on the tooth were removed with a piece of gauze. The tooth was then air-dried, and the sealants were evaluated using a dental explorer for retention, marginal adaptation and marginal discoloration (Fig. 2).

**Table 1.** Modified United States Public Health Service (USPHS) clinical rating system

| Category | Score | Characteristic | Method |
|---|---|---|---|
| Retention | Oscar | the sealant is harmonious and continuous, with an occlusal form and structure | visual/explorer |
| | Alpha | change in the occlusal form of the sealant; all pits and fissures remain covered | visual/explorer |
| | Bravo | loss of sealant from 1 or 2 pits or accessory grooves (partial loss); repair or replacement of the sealant not required | visual/explorer |
| | Charlie | loss of sealant from pits or accessory grooves (partial loss); repair or replacement of the sealant required | visual/explorer |
| | Delta | loss of sealant from all pits (total loss) | visual/explorer |
| Marginal adaptation | Alpha | restoration is fully intact, with no evidence of explorer catch | visual/explorer |
| | Bravo | slight explorer catch in no more than 1/3 of the restoration margin | visual/explorer |
| | Charlie | explorer catch and or penetration evident in more than 1/3 of the restoration margin | visual/explorer |
| Marginal discoloration | Alpha | no visual evidence of discoloration | visual |
| | Bravo | slight discoloration, which can be removed through polishing | visual |
| | Charlie | discoloration extending in a pulpal direction | visual |

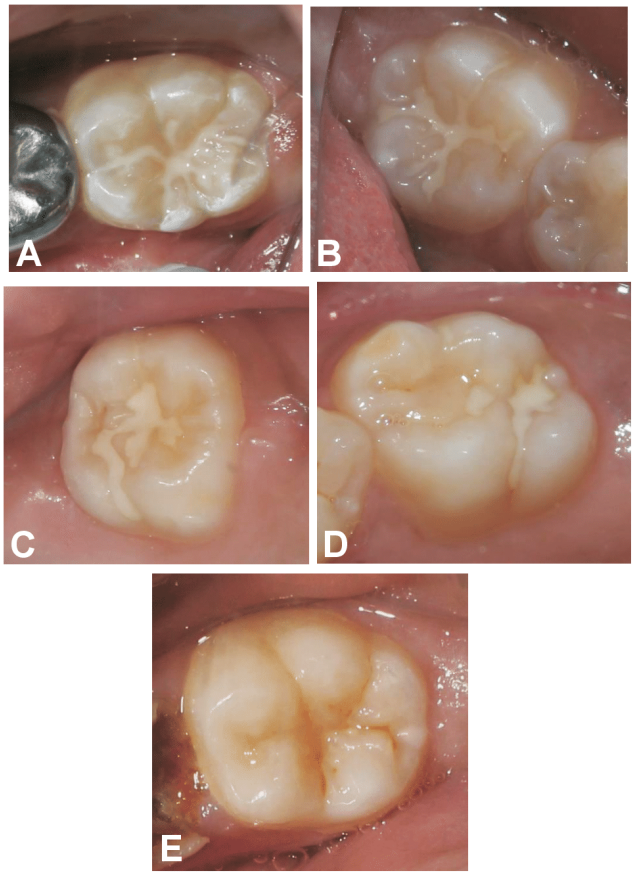


Fig. 2. Assessment of sealant retention

A. Oscar: the sealant is continuous with the occlusal form and structure; B. Alpha: a change in the occlusal form of the sealant is observed, but all pits and fissures remain covered; C. Bravo: sealant loss from 1 or 2 pits or accessory grooves; D. Charlie: sealant loss from more than 2 pits or accessory grooves; E. Delta: sealant loss from all pits (total loss)

## Statistical analysis

The data scores for retention, marginal adaptation and marginal discoloration were expressed as counts with percentages and analyzed using the Windows-based MedCalc Statistical Software v. 18.1.1 (MedCalc Software Ltd., Ostend, Belgium; https://www.medcalc.org).

The retention, marginal discoloration and adaptation scores within each group were analyzed using the Friedman test. The differences between the groups were analyzed using post hoc Mann–Whitney *U* tests. All tests were two-sided, with an alpha set at 0.05. The inter-examiner variability was evaluated using Cohen's kappa test.

# Results

The study was initiated with a total of 55 children, comprising 70 sealants in each of the 2 experimental groups (Group A and Group B). The mean age of the children was 7 years. Five subjects with 10 sealants in each group were lost to follow-up at 12 months. At 12 months, kappa agreement values for retention, marginal adaptation and marginal discoloration were 0.914, 0.918 and 0.936, respectively. These scores indicate almost perfect agreement between the examiners.

## Within-group analysis

Statistically significant differences ($p < 0.0001$) were observed between the baseline and 12-month time points for retention, marginal adaptation and marginal discoloration in both groups (Table 2).

Table 2. Within-group analysis of retention, marginal adaptation and marginal discoloration

| Variable | Hydrophilic sealant (UltraSeal® XT Hydro) | | Hydrophobic sealant (Conseal F) | |
|---|---|---|---|---|
| | $\chi^2$ | *p*-value | $\chi^2$ | *p*-value |
| Retention | 187.420 | <0.0001* | 175.967 | <0.0001* |
| Marginal adaptation | 86.447 | <0.0001* | 51.674 | <0.0001* |
| Marginal discoloration | 58.828 | <0.0001* | 24.500 | <0.0001* |

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

## Between-group analysis

With regard to retention, no significant differences were found at baseline, 1 month and 3 months between hydrophilic and hydrophobic sealants. However, at the 6-month follow-up, the hydrophobic sealants showed significantly better retention compared to the hydrophilic sealants ($p = 0.024$). At the 12-month follow-up, the hydrophobic sealants continued to show significantly better retention compared to the hydrophilic sealants ($p = 0.001$). Notably, 10% of the sealants in the hydrophilic group and 1.7% in the hydrophobic group were completely lost and required replacement at the 12-month follow-up. This finding highlights the superior retention of hydrophobic sealants (Table 3).

The survival rates of the sealants were assessed at baseline and at 1, 3, 6, and 12 months. All sealants showing retention scores of Oscar, Alpha and Bravo were classified as surviving, whereas retention scores of Charlie and Delta indicated loss. A total of 96.7% of sealants in the hydrophobic group and 90% in the hydrophilic group demonstrated survival over the 12-month period (Fig. 3).

With regard to marginal adaptation, no significant difference was observed between the 2 groups up to the 3-month follow-up. However, at the 6-month ($p = 0.015$) and 12-month ($p = 0.023$) follow-ups, the hydrophobic sealants demonstrated significantly better marginal adaptation compared to the hydrophilic sealants (Table 4).

**Table 3.** Comparison of retention scores between Group A and Group B

| Group | Score | Baseline | 1 month | 3 months | 6 months | 12 months |
|---|---|---|---|---|---|---|
| Group A (hydrophilic sealant, UltraSeal XT Hydro) *n* (%) | Oscar | 60 (100) | 50 (83.3) | 26 (43.3) | 6 (10.0) | 0 (0.0) |
| | Alpha | 0 (0.0) | 6 (10.0) | 21 (35.0) | 22 (36.7) | 24 (33.3) |
| | Bravo | 0 (0.0) | 4 (6.7) | 13 (21.7) | 32 (53.3) | 34 (56.7) |
| | Charlie | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 6 (10.0) |
| | Delta | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Group B (hydrophobic sealant, Conseal F) *n* (%) | Oscar | 60 (100) | 53 (88.3) | 33 (55.0) | 9 (15.0) | 3 (5.0) |
| | Alpha | 0 (0.0) | 6 (10.0) | 19 (31.7) | 32 (53.3) | 34 (56.7) |
| | Bravo | 0 (0.0) | 1 (1.7) | 8 (13.3) | 19 (31.7) | 24 (35.0) |
| | Charlie | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (1.7) |
| | Delta | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (1.7) |
| Mann–Whitney *U* test | *U* | 1800.000 | 1701.000 | 1550.500 | 1407.000 | 1234.000 |
| | *Z* | 0.000 | −0.858 | −1.430 | −2.264 | −3.296 |
| | *p*-value | 1.000 | 0.391 | 0.153 | 0.024* | 0.001* |

* statistically significant ($p < 0.05$, Mann–Whitney *U* test).

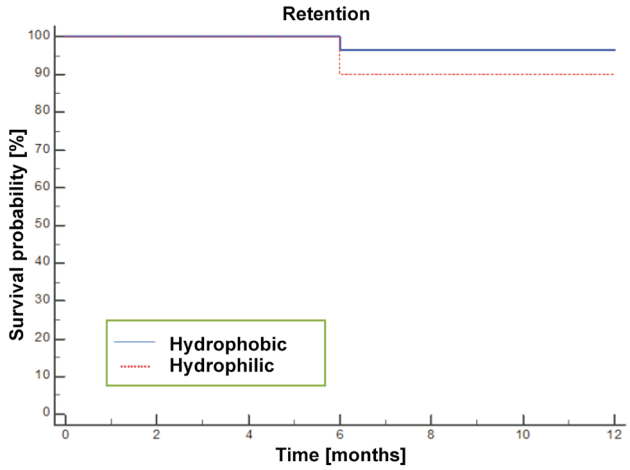


**Fig. 3.** Survival probability of sealants in Group A (UltraSeal® XT Hydro) and Group B (Conseal F)

Regarding marginal discoloration, hydrophilic sealants showed significantly greater discoloration than hydrophobic sealants at the 1-, 3- and 6-month follow-ups. By the 12-month follow-up, 85.0% of sealants in the hydrophobic group were free of marginal discoloration, whereas only 61.7% of sealants in the hydrophilic group remained free of marginal discoloration. A statistically significant difference was observed in favor of hydrophobic sealants when marginal discoloration was compared between the 2 groups at the 12-month follow-up ($p = 0.004$) (Table 5).

## Discussion

The efficacy of pit and fissure sealants in preventing caries is dependent upon their retention and marginal adaptation. Therefore, the objective of this study was to compare the effectiveness of hydrophilic (UltraSeal XT Hydro) and hydrophobic (Conseal F) sealants with respect to retention, marginal adaptation and marginal discoloration.

**Table 4.** Comparison of marginal adaptation scores between Group A and Group B

| Group | Score | Baseline | 1 month | 3 months | 6 months | 12 months |
|---|---|---|---|---|---|---|
| Group A (hydrophilic sealant, UltraSeal XT Hydro) *n* (%) | Alpha | 60 (100) | 50 (83.3) | 41 (68.3) | 31 (51.7) | 27 (45.0) |
| | Bravo | 0 (0.0) | 10 (16.7) | 19 (31.7) | 29 (48.3) | 33 (55.0) |
| | Charlie | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Group B (hydrophobic sealant, Conseal F) *n* (%) | Alpha | 60 (100) | 55 (91.7) | 50 (83.3) | 44 (73.3) | 40 (66.7) |
| | Bravo | 0 (0.0) | 5 (8.3) | 10 (16.7) | 16 (26.7) | 19 (31.7) |
| | Charlie | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (1.7) |
| Mann–Whitney *U* test | *U* | 1800.000 | 1650.000 | 1530.000 | 1410.000 | 1426.500 |
| | *Z* | 0.000 | −1.374 | −1.911 | −2.441 | −2.272 |
| | *p*-value | 1.000 | 0.169 | 0.056 | 0.015* | 0.023* |

* statistically significant ($p < 0.05$, Mann–Whitney *U* test).

Table 5. Comparison of marginal discoloration scores between Group A and Group B

| Group | Score | Baseline | 1 month | 3 months | 6 months | 12 months |
|---|---|---|---|---|---|---|
| Group A (hydrophilic sealant, UltraSeal XT Hydro) *n* (%) | Alpha | 60 (100) | 53 (88.3) | 46 (76.7) | 41 (68.3) | 37 (61.7) |
| | Bravo | 0 (0.0) | 7 (11.7) | 14 (23.3) | 19 (31.7) | 23 (38.3) |
| | Charlie | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Group B (hydrophobic sealant, Conseal F) *n* (%) | Alpha | 60 (100.0) | 59 (98.3) | 56 (93.3) | 53 (88.3) | 51 (85.0) |
| | Bravo | 0 (0.0) | 1 (1.7) | 4 (6.7) | 7 (11.7) | 9 (15.0) |
| | Charlie | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Mann–Whitney *U* test | *U* | 1800.000 | 1620.000 | 1500.000 | 1440.000 | 1380.000 |
| | *Z* | 0.000 | −2.187 | −2.546 | −2.648 | −2.878 |
| | *p*-value | 1.000 | 0.029* | 0.011* | 0.008* | 0.004* |

* statistically significant ($p < 0.05$, Mann–Whitney *U* test).

The results of this study demonstrate that Conseal F showed significantly better retention compared to UltraSeal XT Hydro at both the 6-month and 12-month follow-ups. By the end of the 12-month period, the failure rate of UltraSeal XT Hydro was found to be higher (10%) than that of Conseal F (3.3%). According to a review of published data on sealants, a loss rate of 5–10% per year is generally expected. The failure rate observed in this study is consistent with the expected range.[15]

The lower retention rate of UltraSeal XT Hydro can be attributed to a number of factors. One potential explanation could be its filler content, which is 53%, compared to only 7% in Conseal F.[16,10] Studies by Handelman et al. and Barrie et al. have shown that unfilled sealants tend to exhibit the improved retention compared to filled sealants.[17,18] Another factor could be the reduced penetration depth of the UltraSeal XT Hydro sealant. In a study by Gawali et al., UltraSeal XT Hydro showed significantly reduced penetration compared to the hydrophobic sealant (Fissurit).[19] Additionally, Eliades et al. conducted a laboratory study which demonstrated that hydrophobic sealants exhibit superior sealing characteristics. Hydrophilic sealants, while offering improved setting properties, have lower flow, which affects their ability to penetrate fissures.[20]

The higher retention rates observed with Conseal F in this study may also be attributed to the application of a bonding agent prior to the pleacement of sealants. Studies by Hitt and Feigal and Asselin et al. have reported that the use of a bonding agent prior to the placement of sealants results in enhanced bond strength and a reduction in microleakage when compared to instances where a bonding agent is not used.[21,22]

The results of this study are similar to those of Schlueter et al. and Mohapatra et al., who reported significantly better retention of hydrophobic sealants in comparison with hydrophilic sealants after 1 year.[9,23] In contrast, studies conducted by Khatri et al., Bhatia et al. and Bhat et al. reported a better retention rate of hydrophilic sealants compared to hydrophobic sealants.[11,24,25] Prabakar et al. demonstrated that hydrophilic UltraSeal XT Hydro exhibited enhanced sealant coverage (retention) relative to the conventional Clinpro™ Sealant.[26]

Alongside retention, the marginal adaptation of the sealant is important for its clinical effectiveness. Sealants with poor adaptation can create plaque-retentive sites. Several factors contribute to poor marginal adaptation, including viscosity, polymerization shrinkage, which can result in tensions at the tooth/sealant interface, the formation of marginal microcracks, and ultimately, flaws in the adhesion of the material to the tooth structure.[27]

In the present study, Conseal F exhibited significantly better marginal adaptation compared to UltraSeal XT Hydro at the 6- and 12-month follow-ups. This difference in adaptation may be attributed to the higher viscosity of UltraSeal XT Hydro compared to Conseal F. A study by Mehrabkhani et al. concluded that low-viscosity sealants had significantly better marginal adaptation ($p < 0.002$) in comparison to high-viscosity sealants.[28]

Marginal discoloration of a sealant is often considered an early indicator of its loss of marginal integrity with the adjacent tooth structure. The discoloration is a consequence of marginal breakdown, resulting in a rough and irregular surface. This can create sites for the accumulation of plaque and food debris, as well as facilitate the penetration of oral fluids, which may lead to microleakage and secondary caries formation. If marginal discoloration extends into the margins of the sealant toward the pulp, it should be thoroughly examined with radiographs for potential secondary caries.[29] The UltraSeal XT Hydro sealant showed a significantly higher incidence of marginal discoloration at 1, 3, 6, and 12 months. However, none of these discolorations extended toward the pulp, and they disappeared after polishing.

This study is one of the few to compare both hydrophilic (UltraSeal XT Hydro) and hydrophobic (Conseal F) sealants using a split-mouth study design over a period of 12 months. A randomized split-mouth design was selected to control factors such as patient behavior, diet and oral hygiene, which could influence sealant retention.[30] Additionally, 120 first permanent

molars were randomly assigned to the study groups, with 30 maxillary and 30 mandibular molars in each group. This approach helped to control retention variations resulting from the observed anatomical differences in these teeth.

When sealants are applied in children with a high risk of caries, a review of sealant retention should be part of the recall visits. It is recommended that the recall interval for these children does not exceed 12 months.[31] In this study, which included children with a high risk of caries, recalls were conducted at 1, 3, 6, and 12 months to assess the effectiveness and retention of pit and fissure sealants.

Visual and tactile examinations were employed in this study to evaluate the retention, marginal adaptation and discoloration. This approach may introduce subjective variability in the evaluation, which could be a limitation of the current study. The use of additional methods of evaluation, such as standardized photographs or computer-based software programs, could provide a more objective assessment of these variables.

Accordingly, further studies with extended follow-up periods and more objective assessment methods are required to assess the effectiveness of these sealants.

# Conclusions

The findings of this study indicate that the retention and marginal adaptation of Conseal F (hydrophobic sealant) were significantly better compared to those of UltraSeal XT Hydro (hydrophilic sealant) over a follow-up period of 1 year. Thus, both the null and alternative hypotheses were rejected. The marginal discoloration of Conseal F was significantly higher than that of UltraSeal XT Hydro. However, in both groups, the discoloration could be removed by polishing. Therefore, Conseal F demonstrated superior properties compared to UltraSeal XT Hydro in this study.

## Ethics approval and consent to participate

The study was approved by the Institutional Review Board (Terna Dental College, Navi Mumbai, India; reference No. TDC/IRB-EC/145/2017). Written informed consent was obtained from parents before the procedure.

## 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

Sanjana Ghadge https://orcid.org/0000-0002-3096-2695
Farhin Katge https://orcid.org/0000-0001-8139-6315
Manohar Poojari https://orcid.org/0000-0002-6094-8608
Khushboo Jain https://orcid.org/0000-0002-2764-2586
Vamsi Krishna Chimata https://orcid.org/0000-0003-3561-0282


## References


1. Ripa LW. Occlusal sealing: Rationale of the technique and historical review. *J Am Soc Prev Dent*. 1973;3(1):32–39. PMID:4620519.
2. Carvalho JC, Thylstrup A, Ekstrand KR. Results after 3 years of non-operative occlusal caries treatment of erupting permanent first molars. *Community Dent Oral Epidemiol*. 1992;20(4):187–192. doi:10.1111/j.1600-0528.1992.tb01713.x
3. Grewal H, Verma M, Kumar A. Prevalence of dental caries and treatment needs in the rural child population of Nainital District, Uttaranchal. *J Indian Soc Pedod Prev Dent*. 2009;27(4):224–226. doi:10.4103/0970-4388.57657
4. Feigal RJ, Donly KJ. The use of pit and fissure sealants. *Pediatr Dent*. 2006;28(2):143–150. PMID:16708789.
5. Simonsen RJ. Pit and fissure sealants: Review of the literature. *Pediatr Dent*. 2002;24(5):393–414. PMID:12412954.
6. Kitchens DH. The economics of pit and fissure sealants in preventive dentistry: A review. *J Contemp Dent Pract*. 2005;6(3):95–103. PMID:16127477.
7. Karlzén-Reuterving G, van Dijken JW. A three-year follow-up of glass ionomer cement and resin fissure sealants. *ASDC J Dent Child*. 1995;62(2):108–110. PMID:7608367.
8. Wenderoth CJ, Weinstein M, Borislow AJ. Effectiveness of a fluoride-releasing sealant in reducing decalcification during orthodontic treatment. *Am J Orthod Dentofacial Orthop*. 1999;116(6):629–634. doi:10.1016/s0889-5406(99)70197-6
9. Schlueter N, Klimek J, Ganss C. Efficacy of a moisture-tolerant material for fissure sealing: A prospective randomised clinical trial. *Clin Oral Investig*. 2013;17(3):711–716. doi:10.1007/s00784-012-0740-2
10. Güçlü ZA, Dönmez N, Hurt AP, Coleman NJ. Characterisation and microleakage of a new hydrophilic fissure sealant – UltraSeal XT® hydro™. *J Appl Oral Sci*. 2016;24(4):344–351. doi:10.1590/1678-775720160010
11. Khatri SG, Samuel SR, Acharya S, Patil S, Madan K. Retention of moisture-tolerant and conventional resin-based sealant in six- to nine-year-old children. *Pediatr Dent*. 2015;37(4):366–370. PMID:26314605.
12. Graciano KP, Moysés MR, Ribeiro JC, Pazzini CA, Melgaço CA, Ramos-Jorge J. One-year clinical evaluation of the retention of resin and glass ionomer sealants on permanent first molars in children. *Braz J Oral Sci*. 2015;14(3):190–194. doi:10.1590/1677-3225v14n3a03
13. Erbas Unverdi G, Atac SA, Cehreli ZC. Effectiveness of pit and fissure sealants bonded with different adhesive systems: A prospective randomized controlled trial. *Clin Oral Investig*. 2017;21(7):2235–2243. doi:10.1007/s00784-016-2016-8
14. Cvar JF, Ryge G. Reprint of criteria for the clinical evaluation of dental restorative materials. 1971. *Clin Oral Investig*. 2005;9(4):215–232. doi:10.1007/s00784-005-0018-z
15. Feigal RJ. Sealants and preventive restorations: Review of effectiveness and clinical changes for improvement. *Pediatr Dent*. 1998;20(2):85–92. PMID:9566011.
16. Prabakar J, John J, Arumugham IM, Kumar RP, Sakthi DS. Comparative evaluation of the viscosity and length of resin tags of conventional and hydrophilic pit and fissure sealants on permanent molars: An in vitro study. *Contemp Clin Dent*. 2018;9(3):388–394. doi:10.4103/ccd.ccd_131_18
17. Handelman SL, Leverett DH, Espeland M, Curzon J. Retention of sealants over carious and sound tooth surfaces. *Community Dent Oral Epidemiol*. 1987;15(1):1–5. doi:10.1111/j.1600-0528.1987.tb00470.x
18. Barrie AM, Stephen KW, Kay EJ. Fissure sealant retention: A comparison of three sealant types under field conditions. *Community Dent Health*. 1990;7(3):273–277. PMID:2150181.
19. Gawali PN, Chaugule VB, Panse AM. Comparison of microleakage and penetration depth between hydrophilic and hydrophobic sealants in primary second molar. *Int J Clin Pediatr Dent*. 2016;9(4):291–295. doi:10.5005/jp-journals-10005-1380

20. Eliades A, Birpou E, Eliades T, Eliades G. Self-adhesive restoratives as pit and fissure sealants: A comparative laboratory study. *Dent Mater*. 2013;29(7):752–762. doi:10.1016/j.dental.2013.04.005
21. Hitt JC, Feigal RJ. Use of a bonding agent to reduce sealant sensitivity to moisture contamination: An in vitro study. *Pediatr Dent*. 1992;14(1):41–46. PMID:1502115.
22. Asselin ME, Fortin D, Sitbon Y, Rompré PH. Marginal microleakage of a sealant applied to permanent enamel: Evaluation of 3 application protocols. *Pediatr Dent*. 2008;30(1):29–33. PMID:18402096.
23. Mohapatra S, Prabakar J, Indiran MA, Kumar RP, Sakthi DS. Comparison and evaluation of the retention, cariostatic effect, and discoloration of conventional Clinpro 3M ESPE and hydrophilic Ultraseal XT Hydro among 12–15-year-old schoolchildren for a period of 6 months: A single-blind randomized clinical trial. *Int J Clin Pediatr Dent*. 2020;13(6):688–693. doi:10.5005/jp-journals-10005-1859
24. Bhatia MR, Patel AR, Shirol DD. Evaluation of two resin based fissure sealants: A comparative clinical study. *J Indian Soc Pedod Prev Dent*. 2012;30(3):227–230. doi:10.4103/0970-4388.105015
25. Bhat PK, Konde S, Raj SN, Kumar NC. Moisture-tolerant resin-based sealant: A boon. *Contemp Clin Dent*. 2013;4(3):343–348. doi:10.4103/0976-237X.118394
26. Prabakar J, John J, Arumugham IM, Kumar RP, Srisakthi D. Comparative evaluation of retention, cariostatic effect and discoloration of conventional and hydrophilic sealants – A single blinded randomized split mouth clinical trial. *Contemp Clin Dent*. 2018;9(Suppl 2):S233–S239. doi:10.4103/ccd.ccd_132_18
27. Ramos RP, Chimello DT, Chinelatti MA, Dibb RG, Mondelli J. Effect of three surface sealants on marginal sealing of Class V composite resin restorations. *Oper Dent*. 2000;25(5):448–453. PMID:11203855.
28. Mehrabkhani M, Mazhari F, Sadeghi S, Ebrahimi M. Effects of sealant, viscosity, and bonding agents on microleakage of fissure sealants: An in vitro study. *Eur J Dent*. 2015;9(4):558–563. doi:10.4103/1305-7456.172631
29. McCune RJ, Horowitz HS, Heifetz SB, Cvar J. Pit and fissure sealants: One-year results from a study in Kalispell, Montana. *J Am Dent Assoc*. 1973;87(6):1177–1180. doi:10.14219/jada.archive.1973.0583
30. Lesaffre E, Philstrom B, Needleman I, Worthington H. The design and analysis of split-mouth studies: What statisticians and clinicians should know. *Stat Med*. 2009;28(28):3470–3482. doi:10.1002/sim.3634
31. Ramos-Gomez F, Crystal YO, Ng MW, Tinanoff N, Featherstone JD. Caries risk assessment, prevention, and management in pediatric dental care. *Gen Dent*. 2010;58(6):505–517. PMID:21062720.

Original paper

# Assessment of sleep quality in patients with orofacial pain and headache complaints: A polysomnographic study

Sylwia Orzeszek[1,A–D,F], Helena Martynowicz[2,A–E], Joanna Smardz[1,A–E], Anna Wojakowska[2,B,D,E], Wojciech Bombała[3,C–E], Grzegorz Mazur[2,A,C,D,F], Mieszko Wieckiewicz[1,A–E]

[1] Department of Experimental Dentistry, Outpatient Clinic for Temporomandibular Disorders, Wroclaw Medical University, Poland
[2] Clinical Department of Internal and Occupational Diseases, Hypertension and Clinical Oncology, Wroclaw Medical University, Poland
[3] Statistical Analysis Centre, Wroclaw Medical University, Poland

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(4):549–562

**Address for correspondence**
Sylwia Orzeszek
E-mail: sylwia.winiewska87@gmail.com

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on September 24, 2023
Reviewed on December 6, 2023
Accepted on December 14, 2023

Published online on June 4, 2024

## Abstract

**Background.** Sleep is a physiological function essential for survival, recovery, tissue repair, memory consolidation, and brain function. Pain is also an indispensable aspect of human life. The coexistence of sleep disorders and pain is often described in the literature, yet it is critical to define sleep not only subjectively but also using objective instrumental methods, such as polysomnography, that provide data on sleep quality.

**Objectives.** The aim of the study was to determine the relationship between orofacial pain (OFP), headache (HA) and sleep quality using subjective and objective sleep quality assessment methods. Additionally, we aimed to explore whether poor sleep quality was related to OFP and HA alone or was influenced by the coexistence of psycho-emotional factors such as depression, anxiety and stress.

**Material and methods.** A single-night video-polysomnography was performed on patients from the Outpatient Clinic for Temporomandibular Disorders at Wroclaw Medical University, Poland, who had been diagnosed with OFP and HA. Additionally, questionnaires were employed to assess sleep quality, pain, HA, and the psycho-emotional state.

**Results.** There was no statistically significant relationship between the severity of OFP and HA and polysomnographic sleep quality parameters. On the other hand, the quality of sleep as determined by questionnaire studies correlated markedly with the severity of experienced pain. The severity of pain was found to be significantly correlated with depression, anxiety and perceived stress scores.

**Conclusions.** The psycho-emotional aspects are of critical importance in the perception of OFP and HA. They can be associated with worsened subjective sleep quality, insomnia or daytime sleepiness. Therefore, the treatment of such patients must be preceded by a comprehensive assessment of their psycho-emotional state, as anxiety, stress and depression can significantly influence the course of the disease and the response to treatment procedures.

**Keywords:** sleep quality, polysomnography, headache, orofacial pain, psycho-emotional state

**Cite as**
Orzeszek S, Martynowicz H, Smardz J, et al. Assessment of sleep quality in patients with orofacial pain and headache complaints: A polysomnographic study. *Dent Med Probl.* 2024;61(4):549–562. doi:10.17219/dmp/177008

**DOI**
10.17219/dmp/177008

# Introduction

Sleep is a physiological function essential for recovery from fatigue, tissue repair, memory consolidation, and brain function.[1] It is an active neurobehavioral state that is maintained through a highly organized interaction of neurons and neural circuits in the central nervous system (CNS).[1] Pain is an indispensable part of human life, acting as a physical and emotional signal of body damage that strongly motivates human behavior.[1] Orofacial pain (OFP) encompasses a heterogeneous group of conditions, including dental, mucosal, musculoskeletal, neurovascular, and neuropathic pain,[1] and can be classified in terms of acuity and chronicity. However, the definition of chronic pain, which is pain that persists for more than 3 months, is not useful for assessing OFP and headache (HA) for chronicity.[2–4] In OFP and HA, chronic pain is defined as pain that occurs more than 15 days per month and lasts more than 4 hours daily for at least the preceding 3 months.[2]

Disorders of systems regulating pain and sleep can have a broad negative impact on health and well-being. Sleep complaints are present in 67–88% of individuals suffering from chronic pain disorders.[5–7] Moreover, at least 50% of those with insomnia, the most commonly diagnosed sleep disorder, suffer from chronic pain.[8,9] Several studies that described a strong correlation between pain occurrence and decreased sleep quality also highlighted the crucial role of the psycho-emotional state of patients, including depression, somatization and anxiety.[10–15] A number of studies investigating the relationship between OFP (especially pain associated with temporomandibular disorders (TMD)) and HA and the deterioration of sleep quality demonstrated an association between sleep disturbances and mood swings, impaired memory and cognition, changes in the immune system, and somatic pain complaints.[1,2,9,16]

Previous studies have indicated the existence of a two-way relationship between pain and sleep quality. Sleep disorders can impair regenerative physiological processes and functions that support homeostasis. These disorders contribute to the development and persistence of chronic pain and poorer patient response to treatment, which makes pain management more difficult.[17,18] Other studies have shown that pain can negatively affect sleep via cortical arousal mechanisms that interfere with falling asleep and maintaining sleep.[19] In contrast with acute pain, in which the relationship is linear and rapidly reversible, chronic pain has been described as a vicious cycle with mutual deleterious influences between disturbed sleep and pain.[20] Many authors have also considered the possibility that the pain itself does not cause sleep quality deterioration in patients with OFP but rather psycho-emotional disturbance, such as high levels of stress, anxiety and depression, which are very common in patients suffering from pain.

The psycho-emotional state is a vital factor in the deterioration of sleep quality.[21,22] However, the majority of existing research used subjective sleep quality indicators, such as questionnaires, which inadequately assess sleep quality. Polysomnography is a reliable and objective method for evaluating sleep quality. The objective of this study was to assess the relationship between pain, HA and sleep quality through the use of both subjective and objective assessment methods. All patients underwent a single-night video-polysomnography, and the data was compared to the results of sleep quality questionnaires. The study also aimed to explore whether low sleep quality was solely related to pain and HA, or whether it was influenced by the coexistence of psycho-emotional factors such as depression, anxiety and stress.

# Material and methods

## Participants

The study participants were patients of the Outpatient Clinic for Temporomandibular Disorders at Wroclaw Medical University in Poland. Patients who reported OFP and/or HA and/or impaired sleep quality were referred for a single-night video-polysomnography at the Clinic of Internal Medicine, Occupational Diseases, Hypertension and Clinical Oncology at Wroclaw Medical University, where, in addition to undergoing a polysomnographic examination, they completed several questionnaires related to their psycho-emotional state, sleep quality and pain. The study group consisted of patients who reported primary HA and OFP. Primary HA was defined as pain occurring in temporal relationship to the onset of pain, with no underlying pathological process, disease or traumatic injury. This included migraine, tension-type HA and trigeminal autonomic cephalalgias.[3] Orofacial pain encompasses myofascial OFP (pain localized to the masticatory muscles, with or without functional impairment), temporomandibular joint (TMJ) pain (pain localized to the TMJ, occurring at rest or during jaw movement or palpation) and OFP that resembles presentations of primary HA (pain in the orofacial area resembling one of the primary HA types in pain character, duration and intensity, with or without the associated symptoms of these HA types but without concomitant HA).[4] The study protocol was approved by the Ethical Committee of Wroclaw Medical University (approval No. KB-794/2019). All study participants were informed of the purpose of the study and consented to participate. The study was conducted in accordance with the Declaration of Helsinki for research involving human subjects. The study was registered in the ClinicalTrials.gov database (identifier No. NCT04214561).

## Inclusion criteria

The study included individuals over the age of 18 who reported OFP and/or HA and/or impaired quality of sleep and who were willing to participate.

## Exclusion criteria

The main exclusion criteria were addiction to a drug or medication, the use of medicines that significantly affect the function of the nervous and muscular systems, severe systemic diseases, severe mental disorders, including significant mental disabilities, less than 4 hours of sleep recorded using polysomnography, and lack of consent to participate. Pregnant women, patients with a diagnosed sleep apnea disorder and those using a mandibular advancement device (MAD) or continuous positive airway pressure (CPAP) were excluded from the study.

## Video-polysomnography procedure

The patients underwent a single-night video-polysomnography examination in the Sleep Laboratory at Wroclaw Medical University using a Nox A1s™ (Nox Medical, Reykjavík, Iceland) device. The recording was performed between 10.00 pm and 6.00 am, taking into account the patient's individual preferences and sleeping habits. The polysomnographic examinations included electroencephalography, electrocardiography, electrooculography, and electromyography recordings from the chin area and bilaterally from the regions of the masseter muscles, motion recording of abdominal and thoracic breathing activity, assessment of body position, and audio and video recording. A pulse oximeter (NONIN 3150 WristOx 2; Nonin Medical Inc., Plymouth, USA) was used to record oxygen saturation and pulse, while Noxturnal™ software (Nox Medical), developed for sleep recording and analysis, facilitated data analysis.

## Pain assessment

### McGill Pain Questionnaire

The McGill Pain Questionnaire (MPQ) contains 78 words that describe the experience of pain and provides scores that range from 0 (no pain) to 78 (severe pain). Results are considered clinically significant when the total value is greater than 5.[21] A study by Melzack and Katz assessing the multidimensional nature of pain experience demonstrated that the short-form MPQ (SF-MPQ) is a reliable, valid and consistent measurement tool. The SF-MPQ is suitable for use in specific research settings where the time available to obtain information from patients is limited and where the aim is to obtain information that extends beyond the measurement of pain intensity.[23,24]

### Graded Chronic Pain Scale

The Graded Chronic Pain Scale (GCPS) allows for the grading of chronic pain severity, disability score and disability points. The following classifications are provided: Grade 0 – no pain in the previous 6 months; Grade I – low-intensity characteristic pain intensity <50, low disability, with <3 disability points; Grade II – high-intensity characteristic pain intensity ≥50, low disability, with <3 disability points; Grade III – moderately limiting high disability score of 3 to 4 disability points (regardless of characteristic pain intensity); and Grade IV – a severely limiting high disability score of 5 to 6 disability points (regardless of characteristic pain intensity).[25] Sharma et al. reported that the reliability of the one-month GCPS is equal to or better than the six-month version in terms of pain intensity, days of disability, pain interference, chronic pain grade, and high-impact pain.[26] The study demonstrated that the GCPS is a reliable and valid tool for measuring pain intensity and pain interference.[26]

### Headache Impact Test-6

The Headache Impact Test-6 (HIT-6) score ranges from 36 to 78, with a score ≤49 indicating no or little impact of HA on daily activities, 50–55 indicating a slight impact, 56–59 a significant impact, and ≥60 a severe impact. A study demonstrated that the HIT-6 is a reliable and valid instrument for measuring the impact of HA on daily life in both episodic and chronic migraine sufferers. The internal consistency reliability among migraine sufferers was high, ranging between 0.82 and 0.90.[27]

### Migraine Disability Assessment

The Migraine Disability Assessment (MIDAS) involves the summation of the total number of days indicated in questions 1–5. The grading system includes the following: Grade I – little or no disability over 0–5 days; Grade II – mild disability across 6–10 days; Grade III – moderate disability over 11–20 days; and Grade IV – severe disability for more than 21 days.[27] Stewart et al. demonstrated moderately high test-retest reliability of the MIDAS score for HA sufferers and a correlation with clinical judgment on the need for medical care.[28] The MIDAS score is calculated by summing the number of days missed from work or school, days missed from household chores, days spent on non-work activities, and days spent at work or school, as well as days spent on household chores where productivity was reduced by at least 50% over the previous 3 months. The correlation between the MIDAS summary score and an equivalent daily diary score was 0.63, while the group estimate of the MIDAS score was found to be a valid estimate of a rigorous diary-based measure of disability. In addition, the mean and median values for the MIDAS score in a population-based sample of migraine cases were comparable to those of equivalent diary measures.[28]

### Temporomandibular disorder pain screener

Individuals with a score of 3 or above on the TMD pain screener (range 0–7) are predicted to have painful TMD based on the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) and are, therefore, considered to have a positive TMD pain test result. The TMD pain screener demonstrated excellent sensitivity and specificity levels, as well as validity in correctly identifying participants with pain-related TMD (sensitivity of 99%) and healthy controls (specificity of 97%). The short screener correctly identified individuals with symptoms of competing conditions, including non-painful TMJ disorder (specificity of 95%) and HA not related to TMD (specificity of 96%).[29]

## Subjective assessment of sleep quality

### Insomnia Severity Index

The Insomnia Severity Index (ISI) has a minimal score of 0 and a maximal score of 28. A value of up to 10 is considered within the normal range, while a score of 8–14 indicates subthreshold insomnia. A score of 15–21 indicates significant moderate insomnia, while a score of 22–28 is indicative of severe insomnia.[30]

### Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a tool designed to assess sleep quality over a one-month interval. The measure is comprised of 19 individual items, divided into 7 components of sleep quality, which collectively yield a single global score, ranging from 0 to 21. A lower score indicates better sleep quality. A total score of 5 is considered a designated cut-off point for poor sleep quality.[31]

### Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS) is a validated eight-item questionnaire that is used to assess subjective sleepiness. The ESS values range from 0 (unlikely to fall asleep in any situation) to 24 (high chance of falling asleep in all 8 situations). The final ESS score indicates normal sleepiness (1–10), mild daytime sleepiness (11–14), average daytime sleepiness (15–18), or severe daytime sleepiness (>18).[32]

## Psycho-emotional state assessment

### Sense of Stress Questionnaire

The Sense of Stress Questionnaire (KPS) is designed to assess the structure of stress sensations. The questionnaire comprises 27 statements that can be used to calculate an overall score of an individual's generalized stress level. In addition, 3 results related to emotional tension, intrapsychic stress (resulting from a confrontation with oneself) and external stress (resulting from the confrontation of the individual with the burdens perceived in the social and external worlds) are generated. The questionnaire also contains the scale of lies.[33]

### Perceived Stress Scale-10

The Perceived Stress Scale-10 (PSS-10) results range from 0 to 40. Scores between 0 and 13 are indicative of low stress, scores between 14 and 26 indicate moderate stress, and scores between 27 and 40 are indicative of high perceived stress.[34]

### Patient Health Questionnaire-9

Subjects rate the Patient Health Questionnaire-9 (PHQ-9) responses on a scale of 0 to 3 based on the frequency with which a given symptom has manifested in the last 2 weeks. Total scores for the 9 items range from 0 to 27, with a score of 10 or above indicating a high risk of a depressive episode. The higher the score, the greater the risk of depression. Scores are interpreted as follows: no depression (<5); mild depression (5–9); moderate depression (10–14); moderately severe depression (15–19); or severe depression (20).[35]

### Beck Depression Inventory

The Beck Depression Inventory (BDI) is a 21-item self-rated scale that evaluates key symptoms of depression, including mood, pessimism, sense of failure, self-dissatisfaction, guilt, punishment, self-loathing, self-accusation, suicidal ideation, crying, irritability, social withdrawal, indecisiveness, body image change, work difficulty, insomnia, fatigability, loss of appetite, weight loss, somatic preoccupation, and loss of libido. A total score of 0–13 is considered within the minimal range, while 14–19 is indicative of mild depression, 20–28 of moderate depression, and 29–63 of severe depression.[36]

### Beck Anxiety Inventory

The Beck Anxiety Inventory (BAI) score is calculated by summing 21 items and can range between 0 and 63 points. A total score of 0–7 is interpreted as minimal anxiety, 8–15 as mild anxiety, 16–25 as moderate anxiety, and 26–63 as severe anxiety.[37,38]

### Generalized Anxiety Disorder-7

The Generalized Anxiety Disorder-7 (GAD-7) questionnaire consists of 7 items and the GAD-7 score can range from 0 to 21 points. The cut-off points for mild, moderate and severe anxiety are 5, 10 and 15, respectively.[39]

## Statistical analysis

The medical history, questionnaires and polysomnographic data were entered into a database and subjected to statistical analysis.

The non-parametric Kendall's Tau correlation coefficient was employed to assess the relationship between the variables, with the choice of the coefficient being determined by the fact that the variables did not have a normal distribution. A *p*-value for a correlation coefficient below 0.05 was considered statistically significant. The estimation of the sample size (*N*) was conducted using the power.cor function and the genefu package (https://rdrr.io/bioc/genefu/man/power.cor.html). The sample size for the Kendall correlation significance was calculated on the assumption that the expected value of the correlation coefficient would be 0.7 and that the expected width of the confidence interval would be 0.05. The calculated sample size was 22. Therefore, the 114 patients enrolled in the study constitute a sufficient base for the detection of differences and correlations between the examined parameters.

# Results

## Study sample

A total of 114 Caucasian adults (72 females and 42 males), aged 21–71 years (mean (*M*) = 37.67 years), participated in the study.

## Relationship between pain and headache and subjective assessment of sleep quality

The severity of pain experienced by the participants was assessed using 4 independent questionnaires, as illustrated in Table 1. Taking into account the GCPS values, 64 participants reported experiencing low-intensity pain, with none to low pain-related disability (Grade I), 2 participants described their complaints as high-intensity pain without pain-related disability (Grade IIa), 15 stated that the pain they felt was of a high intensity and caused low pain-related disability (Grade IIb), 11 described their pain as a high-intensity pain causing moderately limiting disability (Grade III), while only 2 participants described their pain level as high-severity with severely limiting disability (Grade IV). The remaining participants did not report pain.

The HIT-6 questionnaire results provided information on the impact of HA on functioning at work, school, home, and in various social settings. The results indicated that 48 subjects reported little or no impact of HA on their daily activities, 14 subjects reported a slight impact, 11 individuals reported a significant impact, and a severe HA effect on daily activities was reported by 35 subjects.

Table 1. Questionnaire results on pain severity, impact of pain on daily activities, perceived disability due to pain, sleep quality, insomnia, and daytime sleepiness reported by the participants

| Questionnaire | Result | Subjects *n* (%) |
|---|---|---|
| GCPS | Grade 0 | 20 (17.54) |
| | Grade I | 64 (56.14) |
| | Grade IIa | 2 (1.76) |
| | Grade IIb | 15 (13.16) |
| | Grade III | 11 (9.64) |
| | Grade IV | 2 (1.76) |
| HIT-6 | no or little impact | 48 (44.44) |
| | slight impact | 14 (12.96) |
| | significant impact | 11 (10.19) |
| | severe impact | 35 (32.41) |
| MIDAS | Grade I | 52 (52.00) |
| | Grade II | 10 (10.00) |
| | Grade III | 15 (15.00) |
| | Grade IV | 23 (23.00) |
| SF-MPQ | ≤5 | 74 (64.91) |
| | >5 | 40 (35.09) |
| TMD pain screener | ≤3 | 47 (53.41) |
| | >3 | 41 (46.59) |
| PSQI | ≤5 | 36 (33.03) |
| | >5 | 73 (66.97) |
| ISI | normal | 29 (32.59) |
| | subthreshold insomnia | 33 (37.07) |
| | significant moderate insomnia | 21 (23.60) |
| | severe insomnia | 6 (6.74) |
| ESS | normal | 61 (69.31) |
| | mild daytime sleepiness | 18 (20.46) |
| | average daytime sleepiness | 7 (7.96) |

GCPS – Graded Chronic Pain Scale; HIT-6 – Headache Impact Test-6; MIDAS – Migraine Disability Assessment; SF-MPQ – short-form McGill Pain Questionnaire; TMD – temporomandibular disorders; PSQI – Pittsburgh Sleep Quality Index; ISI – Insomnia Severity Index; ESS – Epworth Sleepiness Scale.

The impact of HA on functioning at work, home and in social settings was assessed using the MIDAS scale. Participants were classified into 4 disability groups based on the number of days they had to limit their activities due to HA. Little or no disability was reported by 52 participants (Grade I), 10 reported mild disability (Grade II), 15 – moderate disability (Grade III), and 23 – severe disability (Grade IV). A clinically significant pain level was reported by 40 participants in the SF-MPQ study. The remaining participants did not report any pain, and the complaints were not clinically significant. At the same time, clinically significant TMD-related pain was recorded for 41 participants who completed the TMD pain screener.

The participants completed questionnaires designed to assess their sleep quality. In the PSQI, the cut-off point indicating poor sleep quality was reached by 73 subjects, while

36 subjects were classified as having normal sleep quality. The ISI indicated that 29 participants had normal sleep quality, 33 individuals had subthreshold insomnia, 21 – significant moderate insomnia, and 6 – severe insomnia. According to the ESS, 61 respondents exhibited no abnormalities, 18 displayed mild daytime sleepiness, 7 had average daytime sleepiness, and 2 exhibited severe daytime sleepiness. Table 1 provides a summary of the aforementioned results.

The Kendall's Tau correlation coefficient showed a statistically significant association between self-reported pain severity and decreased sleep quality. Furthermore, there was a correlation between greater pain and related disability, as assessed using the GCPS, and subjective sleep quality, as assessed using the PSQI and ISI questionnaires. The correlation between the increased pain reported in the SF-MPQ and the worsened sleep quality observed in the ISI and PSQI scores was statistically significant. Participants whose scores exceeded the cut-off point in the TMD pain screener exhibited a deterioration in sleep quality, as indicated by the ISI and PSQI scores. The relationship between the increase in pain complaints reported in the HIT-6 questionnaire and the deterioration in sleep quality observed in the PSQI, ISI and ESS questionnaires was also statistically significant. A similar relationship was observed between the MIDAS questionnaire values and the PSQI, ISI and ESS scores. Consequently, the current research indicates that pain and related disability were the cause of poor sleep quality. Table 2 illustrates the presence of subjective sleep disturbances based on the increase in reported pain.

## Relationship between pain and headache and the assessment of sleep quality in polysomnography

The quality of sleep was also objectively evaluated in a sleep research laboratory. All participants underwent a polysomnographic examination, which recorded the parameters necessary for the objective assessment of sleep quality. A statistical analysis was conducted, including the following polysomnographic parameters: total sleep time (TST), which represents the sum of time spent asleep in minutes; wake after sleep onset (WASO), which refers to waking up after falling asleep; sleep latency (SL), which is defined as the time from turning off the light to falling asleep (and is considered the first occurrence of non-rapid eye movement (NREM) stage 2); and sleep efficiency (SE), which is calculated as TST/total time in bed (TBT) × 100%. Table 3 presents the results of the polysomnographic examination.

**Table 2.** Presence of subjective sleep disturbances based on the increase in pain reported by the participants

| Pair of variables | *n* | Kendall's Tau | *Z* | *p*-value |
|---|---|---|---|---|
| GCPS & PSQI global score | 110 | 0.159381 | 2.468092 | 0.014* |
| GCPS & ISI | 87 | 0.204041 | 2.798379 | 0.005* |
| GCPS & ESS | 87 | –0.007137 | –0.097879 | 0.922 |
| HIT-6 & PSQI global score | 108 | 0.197245 | 3.025658 | 0.002* |
| HIT-6 & ISI | 88 | 0.343241 | 4.735509 | <0.001* |
| HIT-6 & ESS | 85 | 0.254950 | 3.454555 | 0.001* |
| MIDAS & PSQI global score | 101 | 0.248177 | 3.677424 | <0.001* |
| MIDAS & ISI | 82 | 0.410572 | 5.460118 | <0.001* |
| MIDAS & ESS | 81 | 0.164313 | 2.171236 | 0.030* |
| SF-MPQ & PSQI global score | 114 | 0.290926 | 4.588840 | <0.001* |
| SF-MPQ & ISI | 89 | 0.359480 | 4.988756 | <0.001* |
| SF-MPQ & ESS | 88 | 0.138921 | 1.916616 | 0.055 |
| TMD pain screener & PSQI global score | 88 | 0.228828 | 3.157022 | 0.002* |
| TMD pain screener & ISI | 66 | 0.272170 | 3.230833 | 0.001* |
| TMD pain screener & ESS | 87 | 0.042816 | 0.587212 | 0.557 |

* statistically significant ($p < 0.05$, Kendall's Tau correlation).

**Table 3.** Results of polysomnographic examination

| Parameter | *M* | *Me* | Minimum | Maximum | Lower quartile | Upper quartile | Quartile deviation | *SD* |
|---|---|---|---|---|---|---|---|---|
| TST | 434.0883 | 449.0000 | 145.4000 | 530.5000 | 399.5000 | 481.2000 | 81.70000 | 68.62323 |
| WASO | 43.6350 | 29.5000 | 0.5000 | 171.0000 | 17.5000 | 57.0000 | 39.50000 | 38.13132 |
| SL | 19.0922 | 14.9000 | 0.3000 | 68.1000 | 6.6000 | 25.4000 | 18.80000 | 16.06708 |
| SE | 86.2447 | 88.1000 | 59.1000 | 97.9000 | 80.6000 | 93.4000 | 12.80000 | 8.75870 |

TST – total sleep time; WASO – wake after sleep onset; SL – sleep latency; SE – sleep efficiency; *M* – mean; *Me* – median; *SD* – standard deviation.

The results of the Kendall's Tau test were unexpected, as the quality of sleep measured during polysomnography did not show a statistically significant relationship with an increase in the intensity of reported pain and related disability. Only participants whose scores exceeded the cut-off point on the TMD pain screener demonstrated a reduction in the WASO parameter in the polysomnographic examination. The relationship between the increase in reported pain complaints in questionnaire studies and selected parameters assessed in polysomnography are presented in Table 4.

A statistical analysis was conducted to assess the relationship between the SE and SL measured in the polysomnographic examination. The parameters were defined by the participants as 2 of the 7 components of sleep quality in the PSQI questionnaire (C2 falling asleep – analysis of the SL in minutes and the number of nights where the SL was extended to more than 30 min; C4 SE – quantitative assessment of the ratio of TST to TBT). The results of the comparison between the SL and SE are presented in Table 5.

**Table 4.** Relationship between pain complaints reported in questionnaire studies and selected parameters of sleep quality assessed in polysomnography

| Pair of variables | *n* | Kendall's Tau | *Z* | *p*-value |
|---|---|---|---|---|
| TMD pain screener & TST | 80 | –0.019701485 | –0.25865555 | 0.796 |
| TMD pain screener & WASO | 80 | –0.1873126 | –2.45917716 | 0.014* |
| TMD pain screener & SL | 80 | 0.0706424102 | 0.927445358 | 0.354 |
| TMD pain screener & SE | 80 | 0.0693498556 | 0.910475753 | 0.363 |
| HIT-6 & TST | 98 | 0.0455309984 | 0.664223607 | 0.507 |
| HIT-6 & WASO | 98 | –0.102724483 | –1.49858402 | 0.134 |
| HIT-6 & SL | 98 | –0.035229518 | –0.51394168 | 0.607 |
| HIT-6 & SE | 98 | 0.0281436189 | 0.41056987 | 0.681 |
| MIDAS & TST | 92 | 0.114810466 | 1.62095682 | 0.105 |
| MIDAS & WASO | 92 | –0.094433626 | –1.33326548 | 0.182 |
| MIDAS & SL | 92 | 0.0688945252 | 0.972690504 | 0.331 |
| MIDAS & SE | 92 | 0.0321546277 | 0.453976581 | 0.650 |
| GCPS & TST | 100 | 0.0637825268 | 0.940261478 | 0.347 |
| GCPS & WASO | 100 | –0.060060854 | –0.88539778 | 0.376 |
| GCPS & SL | 100 | 0.052715005 | 0.777107635 | 0.437 |
| GCPS & SE | 100 | –0.016318200 | –0.24055766 | 0.810 |

* statistically significant ($p < 0.05$, Kendall's Tau correlation).

**Table 5.** Comparison of sleep latency and sleep efficiency in questionnaire studies and polysomnographic examination

| Pair of variables | *n* | Kendall's Tau | *Z* | *p*-value |
|---|---|---|---|---|
| TMD pain screener & SL | 80 | 0.070642 | 0.927445 | 0.354 |
| TMD pain screener & SE | 80 | 0.069349 | 0.910475 | 0.363 |
| TMD pain screener & PSQI SL | 85 | 0.183346 | 2.48432 | 0.013* |
| TMD pain screener & PSQI habitual SE | 85 | 0.164382 | 2.22736 | 0.026* |
| HIT-6 & SL | 98 | –0.035229 | –0.51394 | 0.607 |
| HIT-6 & SE | 98 | 0.0281436 | 0.410569 | 0.681 |
| HIT-6 & PSQI SL | 107 | 0.053866 | 0.82232 | 0.411 |
| HIT-6 & PSQI habitual SE | 107 | 0.173293 | 2.64551 | 0.008* |
| MIDAS & SL | 92 | 0.068894 | 0.97269 | 0.331 |
| MIDAS & SE | 92 | 0.032154 | 0.45397 | 0.650 |
| MIDAS & PSQI SL | 100 | 0.166755 | 2.45825 | 0.014* |
| MIDAS & PSQI habitual SE | 100 | 0.211255 | 3.11425 | 0.002* |
| GCPS & SL | 100 | 0.052715 | 0.77710 | 0.437 |
| GCPS & SE | 100 | –0.016318 | –0.24055 | 0.810 |
| GCPS & PSQI SL | 107 | 0.019255 | 0.29394 | 0.769 |
| GCPS & PSQI habitual SE | 107 | 0.109976 | 1.67891 | 0.093 |

* statistically significant ($p < 0.05$, Kendall's Tau correlation).

The relationship between sleep quality parameters derived from polysomnography and sleep quality as assessed subjectively by participants in questionnaire studies is summarized in Table 6. The lack of correlation between these parameters confirms that subjectively assessed sleep quality is influenced by factors other than pain, as pain alone did not correlate with the occurrence of sleep disorders in the polysomnographic examination.

## Relationship between the level of anxiety, depression and stress, and the subjective assessment of sleep quality

The PSS-10 and KPS questionnaires assessed perceived stress levels, the BDI and PHQ-9 evaluated depression, and the GAD-7 and BAI measured anxiety. Table 7 presents the number of participants at risk of psycho-emotional disturbances, defined as an increased sense of anxiety, stress and/or depression.

The statistical analysis revealed a significant correlation between the occurrence of pain, its severity and pain-related disability that causes limitations in everyday professional, family and social activities and affects the participants' psycho-emotional state. This relationship is presented in Table 8.

The relationship between subjective sleep disturbances and the psycho-emotional state was significant. Increased anxiety, stress and depression scores correlated with a decrease in subjective sleep quality. Furthermore, a decreased psycho-emotional status was consistently correlated with increased participant complaints of poor sleep

**Table 7.** Stress, anxiety and depression levels reported by the participants

| Questionnaire | Result | Subjects *n* (%) |
|---|---|---|
| PSS-10 | low level of stress | 30 (27.78) |
| | moderate level of stress | 65 (60.19) |
| | high level of stress | 13 (12.04) |
| KPS | STEN 1–4 | 74 (66.67) |
| | STEN 5–6 | 26 (23.42) |
| | STEN 7–10 | 11 (9.91) |
| BDI | no depression | 72 (65.45) |
| | mild depression | 22 (20.00) |
| | moderate depression | 11 (10.00) |
| | severe depression | 5 (4.55) |
| PHQ-9 | no depression | 33 (30.28) |
| | mild depression | 41 (37.61) |
| | moderate depression | 24 (22.02) |
| | moderately severe depression | 8 (7.34) |
| | severe depression | 3 (2.75) |
| GAD-7 | minimal anxiety | 55 (51.89) |
| | mild anxiety | 33 (31.13) |
| | moderate anxiety | 11 (10.38) |
| | severe anxiety | 7 (6.60) |
| BAI | minimal anxiety | 77 (73.33) |
| | moderate anxiety | 16 (15.24) |
| | severe anxiety | 12 (11.43) |

PSS-10 – Perceived Stress Scale-10; KPS – Sense of Stress Questionnaire; BDI – Beck Depression Inventory; PHQ-9 – Patient Health Questionnaire-9; GAD-7 – Generalized Anxiety Disorder-7; BAI – Beck Anxiety Inventory.

**Table 6.** Comparison of sleep quality parameters derived from polysomnography with those obtained in questionnaire studies

| Pair of variables | *n* | Kendall's Tau | *Z* | *p*-value |
|---|---|---|---|---|
| TST & ISI | 81 | 0.091348 | 1.20708 | 0.227 |
| TST & ESS | 81 | 0.074740 | 0.98762 | 0.323 |
| TST & PSQI global score | 103 | 0.101410 | 1.51797 | 0.129 |
| WASO & ISI | 81 | 0.021504 | 0.28415 | 0.776 |
| WASO & ESS | 81 | –0.110765 | –1.46365 | 0.143 |
| WASO & PSQI global score | 103 | 0.008127 | 0.12165 | 0.903 |
| SL & ISI | 81 | 0.104323 | 1.37853 | 0.168 |
| SL & ESS | 81 | –0.068686 | –0.90762 | 0.364 |
| SL & PSQI global score | 103 | 0.0257558 | 0.38553 | 0.700 |
| SE & ISI | 81 | –0.039522 | –0.5222 | 0.601 |
| SE & ESS | 81 | 0.1059903 | 1.40056 | 0.161 |
| SE & PSQI global score | 103 | –0.052512 | –0.7860 | 0.432 |
| GCPS & SL | 100 | 0.052715 | 0.77710 | 0.437 |
| GCPS & SE | 100 | –0.016318 | –0.24055 | 0.810 |
| GCPS & PSQI SL | 107 | 0.019255 | 0.29394 | 0.769 |
| GCPS & PSQI habitual SE | 107 | 0.109976 | 1.67891 | 0.093 |

* statistically significant ($p < 0.05$, Kendall's Tau correlation).

quality (as measured by the PSQI), increased daytime sleepiness (as measured by the ESS) and increased insomnia severity (as measured by the ISI). The relationship was more pronounced than that between perceived pain and the subjective assessment of sleep quality. Therefore, the psycho-emotional state of patients may be a factor influencing their perception of sleep quality (Table 9).

## Relationship between the level of anxiety, depression and stress, and the objective assessment of sleep quality in polysomnography

The analysis of the impact of the psycho-emotional state on sleep quality, as measured using polysomnography, revealed that the relationship between the 2 variables is not straightforward or statistically unambiguous. Patients with higher stress levels (as measured by the KPS) exhibited a shortened TST ($p$ = 0.017), worse SE ($p$ = 0.031), longer SL ($p$ = 0.030), and a longer WASO ($p$ = 0.028). However, this relationship was only found for TST ($p$ = 0.049) when measuring stress levels using the PSS-10. There was no longer a significant correlation with WASO ($p$ = 0.115), SL ($p$ = 0.615) or SE ($p$ = 0.235). The examination of other sleep parameters during polysomnography did not reveal any abnormalities in participants with elevated levels of stress, anxiety or depression. The data is presented in Table 10.

# Discussion

The objective of the study was to determine the relationship between reported OFP, HA and sleep quality using subjective and objective assessment methods. The study also aimed to explore whether the poor quality of sleep was only related to OFP and HA, or whether it was influenced by the coexistence of psycho-emotional factors such as depression, anxiety and stress. Even though the relationship between OFP and HA and the deterioration of sleep quality was reported by patients in questionnaire studies, no significant deviations were found in the objective study using polysomnography despite complaints reported by patients. However, the study revealed that patients who reported reduced sleep quality in questionnaire studies also exhibited elevated levels of stress, anxiety and depression.

**Table 8.** Relationship between the level of anxiety, depression and stress, and pain severity

| Pair of variables | *n* | Kendall's Tau | *Z* | *p*-value |
|---|---|---|---|---|
| TMD pain screener & PSS-10 | 85 | 0.271585 | 3.679949 | <0.001* |
| TMD pain screener & KPS | 87 | 0.245056 | 3.360890 | 0.001* |
| TMD pain screener & BDI | 86 | 0.280451 | 3.823269 | <0.001* |
| TMD pain screener & PHQ-9 | 86 | 0.327406 | 4.463393 | <0.001* |
| TMD pain screener & BAI | 83 | 0.330526 | 4.423432 | <0.001* |
| TMD pain screener & GAD-7 | 83 | 0.370594 | 4.959670 | <0.001* |
| GCPS & PSS-10 | 106 | 0.273809 | 4.159796 | <0.001* |
| GCPS & KPS | 109 | 0.193104 | 2.976261 | 0.003 |
| GCPS & BDI | 108 | 0.250197 | 3.837918 | <0.001* |
| GCPS & PHQ-9 | 108 | 0.273731 | 4.198926 | <0.001* |
| GCPS & BAI | 104 | 0.302987 | 4.558022 | <0.001* |
| GCPS & GAD-7 | 104 | 0.293885 | 4.421089 | <0.001* |
| HIT-6 & PSS-10 | 108 | 0.226315 | 3.471580 | 0.001* |
| HIT-6 & KPS | 107 | 0.300810 | 4.592199 | <0.001* |
| HIT-6 & BDI | 108 | 0.348665 | 5.348384 | <0.001* |
| HIT-6 & PHQ-9 | 107 | 0.330598 | 5.046953 | <0.001* |
| HIT-6 & BAI | 104 | 0.319397 | 4.804873 | <0.001* |
| HIT-6 & GAD-7 | 105 | 0.310308 | 4.691280 | <0.001* |
| MIDAS & PSS-10 | 100 | 0.330638 | 4.874154 | <0.001* |
| MIDAS & KPS | 100 | 0.275152 | 4.056203 | <0.001* |
| MIDAS & BDI | 101 | 0.400234 | 5.930566 | <0.001* |
| MIDAS & PHQ-9 | 101 | 0.332793 | 4.931234 | <0.001* |
| MIDAS & BAI | 99 | 0.377552 | 5.536881 | <0.001* |
| MIDAS & GAD-7 | 99 | 0.328361 | 4.815483 | <0.001* |

* statistically significant ($p < 0.05$, Kendall's Tau correlation).

**Table 9.** Relationship between the level of anxiety, depression and stress, and the subjective assessment of sleep quality

| Pair of variables | *n* | Kendall's Tau | *Z* | *p*-value |
|---|---|---|---|---|
| PSS-10 & PSQI | 108 | 0.324845 | 4.982985 | <0.001* |
| PSS-10 & ISI | 88 | 0.394190 | 5.438427 | <0.001* |
| PSS-10 & ESS | 85 | 0.212189 | 2.875137 | 0.004* |
| KPS & PSQI | 112 | 0.302435 | 4.727067 | <0.001* |
| KPS & ISI | 88 | 0.339918 | 4.689672 | <0.001* |
| KPS & ESS | 87 | 0.200043 | 2.743542 | 0.006* |
| BDI & PSQI | 110 | 0.405912 | 6.285733 | <0.001* |
| BDI & ISI | 88 | 0.428127 | 5.906635 | <0.001* |
| BDI & ESS | 86 | 0.272479 | 3.714598 | <0.001* |
| PHQ-9 & PSQI | 109 | 0.437751 | 6.746935 | <0.001* |
| PHQ-9 & ISI | 87 | 0.480670 | 6.592274 | <0.001* |
| PHQ-9 & ESS | 86 | 0.341903 | 4.661024 | <0.001* |
| BAI & PSQI | 106 | 0.402993 | 6.122401 | <0.001* |
| BAI & ISI | 85 | 0.409755 | 5.552148 | <0.001* |
| BAI & ESS | 83 | 0.192642 | 2.578127 | 0.010* |
| GAD-7 & PSQI | 106 | 0.355888 | 5.406769 | <0.001* |
| GAD-7 & ISI | 86 | 0.428333 | 5.839290 | <0.001* |
| GAD-7 & ESS | 84 | 0.208219 | 2.804021 | 0.005* |

* statistically significant ($p < 0.05$, Kendall's Tau correlation).

**Table 10.** Relationship between the level of anxiety, depression and stress, and the objective assessment of sleep quality in polysomnography

| Pair of variables | *n* | Kendall's Tau | *Z* | *p*-value |
|---|---|---|---|---|
| PSS-10 & TST | 98 | 0.135076 | 1.97055 | 0.049* |
| PSS-10 & WASO | 98 | –0.108172 | –1.57806 | 0.115 |
| PSS-10 & SL | 98 | –0.034472 | –0.50289 | 0.615 |
| PSS-10 & SE | 98 | 0.081371 | 1.18708 | 0.235 |
| KPS & TST | 102 | 0.159898 | 2.38142 | 0.017* |
| KPS & WASO | 102 | –0.147246 | –2.19299 | 0.028* |
| KPS & SL | 102 | –0.145709 | –2.17009 | 0.030* |
| KPS & SE | 102 | 0.1446899 | 2.1549 | 0.031* |
| BDI & TST | 100 | 0.074851 | 1.10343 | 0.270 |
| BDI & WASO | 100 | –0.080055 | –1.18014 | 0.238 |
| BDI & SL | 100 | –0.1006566 | –1.483847 | 0.138 |
| BDI & SE | 100 | 0.08106160 | 1.1949840 | 0.232 |
| PHQ-9 & TST | 99 | 0.072239 | 1.05941 | 0.289 |
| PHQ-9 & WASO | 99 | –0.098424 | –1.44341 | 0.149 |
| PHQ-9 & SL | 99 | 0.0114757 | 0.168293 | 0.866 |
| PHQ-9 & SE | 99 | 0.0490954 | 0.719996 | 0.472 |
| BAI & TST | 96 | 0.106368 | 1.53526 | 0.125 |
| BAI & WASO | 96 | –0.075987 | –1.09676 | 0.273 |
| BAI & SL | 96 | 0.01954793 | 0.282144 | 0.778 |
| BAI & SE | 96 | 0.02843335 | 0.410392 | 0.682 |
| GAD-7 & TST | 98 | 0.125856 | 1.83603 | 0.066 |
| GAD-7 & WASO | 98 | –0.077735 | –1.13403 | 0.257 |
| GAD-7 & SL | 98 | 0.078594 | 1.146563 | 0.252 |
| GAD-7 & SE | 98 | 0.028898 | 0.421578 | 0.673 |

* statistically significant ($p < 0.05$, Kendall's Tau correlation).

Many studies have demonstrated that individuals with severe and prolonged pain and HA exhibit elevated levels of stress, anxiety and depression. These studies also indicated that psycho-social factors play a vital role in predisposing to, and the course of, severe pain or HA and their treatment.[40–42] Such a relationship is in line with the results of the current study, which confirms the crucial role of the psycho-emotional state of patients suffering from pain in the course of the disease and the selection of treatment methods, which should aim to improve patient well-being and include psychological consultations and, if required, psychiatric consultations for support and multi-directional therapy.

Yap et al. examined the effect of the severity of TMD pain on sleep quality and the impact of TMD diagnosis type on sleep disorders.[43] Their findings indicated that individuals with moderate to severe pain had significantly worse sleep than those with mild pain. Participants with pain-related and/or intra-articular TMD reported significantly worse sleep quality than TMD-free controls. In addition, individuals with muscle pain and those with muscle pain and joint pain presented significantly worse sleep than those with non-painful joint disorders. However, in this study, sleep quality was only assessed using the PSQI.[43] Kim and Kim also reported significant differences in global PSQI scores between 3 pain diagnosis groups in Korean TMD patients.[44] Other authors indicated that reduced sleep quality in participants with pain associated with TMD can negatively impact treatment outcomes and quality of life.[45,46] The findings of the cited studies contrast with the results of the current study, which indicate pain as the primary cause of sleep quality abnormalities reported by patients. On the other hand, Yatani et al. demonstrated that poor sleep quality may not be solely attributed to elevated pain severity.[47] Their findings indicated that psychological distress and low perceived life control contributed to reduced sleep quality.[47] A similar conclusion can be drawn from the observations in the current study.

When discussing the issue of pain and sleep quality, it is important to consider the neurobiological aspect of OFP and the neural processes underlying sleep, particularly the mechanisms underlying the interactions between pain and sleep, including sleep disturbances. Chronic pain is often associated with poor sleep quality, which can also be a cause of pain. This interdependence indicates that the reduction of OFP and the improvement of sleep quality should be targeted together.[20] Patients with chronic pain tend to exhibit either short or long sleep durations and also experience depressive mood symptoms.[48,49] According to other authors, chronic pain may increase the risk of insomnia,[50] and insomnia may be associated with a reduction in pain tolerance and lower SE.[51]

Lavigne and Sessle state that, during normal sleep in healthy adults, nociceptive transmission is partially impaired to maintain sleep continuity, resulting in a higher threshold of excitability or a lower rate of response to noxious stimuli in light sleep (stages N1 and N2) and in deep sleep (stage N3), where it is even more important.[20] However, this relationship is variable in REM sleep. These processes ensure that low-intensity stimuli have little or no effect on the quality of sleep if the sleep takes place in good conditions.[20] Consequently, the link between pain and sleep quality, especially chronic pain, is indisputable. However, it must not be forgotten that there are also processes that prevent ailments from affecting the quality and course of sleep.

In their study, Dubrovsky et al. employed double-night polysomnography to assess sleep quality, using Symptom Checklist-90 to evaluate depressive symptoms and the PSQI to assess subjective sleep quality.[52] The findings of their study were comparable to those of the current study. Increased self-reported sleep problems in women with myofascial pain were more attributable to reporting depressive symptoms than to pain intensity or objective polysomnography measures. The authors warned against assuming that myofascial pain is associated with poor sleep quality and highlighted the potential of questionnaire studies such as the PSQI to effectively diagnose the presence of sleep disorders in study participants. Furthermore, the authors advised that more attention should be paid to the necessity of objective sleep quality diagnostics, which can be achieved through polysomnographic examination. This method does not often show deviations from the norm. As such, the poor sleep quality reported by patients suffering from pain may be caused by psycho-emotional disturbances.[52] In contrast, Smith et al. revealed that approx. 36% of participants with TMD pain suffered from insomnia, 28% had sleep apnea, and some patients were also diagnosed with a mild form of sleep-disordered breathing known as respiratory effort-related arousals.[53]

When considering the relationship between pain, sleep quality and the psycho-emotional state, it is important to acknowledge the role of the pituitary hormones pathway. The daily rhythm of cortisol secretion is relatively stable and primarily under the influence of the circadian clock. Moreover, the activity of the hypothalamic–pituitary–adrenal (HPA) axis is modulated by many different factors, of which sleep has a modest but significant impact. Sleep onset has an inhibitory effect on cortisol secretion, while awakenings and sleep offset are associated with cortisol stimulation. During waking, a correlation between cortisol secretory bursts and indices of central arousal has also been detected. A lack of sleep and/or a reduction in sleep quality results in a slight activation of the axis, while sudden changes in sleep duration cause a profound disruption of the circadian rhythm of cortisol.[54] The HPA axis is also vital for stress adaptation, with its activation causing the secretion of glucocorticoids. The HPA stress response is primarily driven by neural mechanisms and involves the release of corticotropin-releasing hormone (CRH) from hypothalamic paraventricular nucleus (PVN) neurons.

The pathways that activate CRH release are stressor dependent. Reactive responses to homeostatic disruption frequently involve direct noradrenergic or peptidergic drive of PVN neurons by sensory relays, whereas anticipatory responses use oligosynaptic pathways originating in upstream limbic structures. These relationships are complex and require further study.[54,55]

Many studies have been conducted on the relationship between pain and sleep disorders. The results of some of these studies are presented in this paper. However, only one of these studies used a subjective assessment of sleep quality, i.e., polysomnography. In the current study, all patients underwent a polysomnographic examination, which enabled a comparison of their reports of poorer sleep quality with the objective sleep quality parameters derived from polysomnography. An additional advantage of this study was the comparison of pain levels and sleep quality reported by patients and sleep parameters from polysomnography with the psycho-emotional state of the patients.

## Limitations

The limitations of the study relate to the use of single-night video-polysomnography and the discrepancy between the sleep laboratory environment and the participant's usual sleeping conditions. The negative impact of the new environment on sleep quality is most pronounced during the first night spent in the sleep laboratory. Another limitation of the current research was the use of questionnaires to diagnose pain and HA, with no instrument used to objectively measure pain severity. Additionally, the analysis of the examined relationships did not account for gender.

# Conclusions

Orofacial pain (OFP resembling presentations of primary HA, myofascial OFP and TMJ pain) and HA are serious and very common problems. Among the difficulties reported by patients dealing with pain, sleep disturbances are frequently observed. As such, the role of sleep disorders in pain patients cannot be neglected, and the possibility of sleep disturbances, sleep apnea, sleep-related hypoxia, and insomnia in patients with OFP or HA should always be considered. However, the results of this study indicate that the poor sleep quality reported by a patient may not be related to sleep disorders as determined by polysomnographic examination. Indeed, long-term pain is frequently associated with the co-occurrence of disorders of the patient's psycho-emotional state, including anxiety, depression and increased stress levels. Therefore, the psycho-emotional state of a patient suffering from pain may be the underlying cause of the subjective deterioration in sleep quality. Consequently, patients with long-lasting pain should also be examined for psycho-emotional disorders and, if indicated, provided with professional care.

Self-reporting of poor sleep quality may be enhanced by the incorporation of polysomnography into the diagnostic process, which should be considered the gold standard for the diagnosis of sleep disorders. Therefore, the identification of comorbidities, a detailed study of the psycho-emotional state of the patient, and questionnaire studies on sleep quality are essential for selecting the most effective management strategy for patients with OFP or HA. The current study found that the presence and intensity of patient-reported OFP and HA reduced sleep quality subjectively, but not objectively. Furthermore, these relationships were impacted by the presence of psycho-emotional disorders. However, the lack of comprehensive data on the mechanisms involved in the interactions between OFP and sleep warrants further research.

## Ethics approval and consent to participate

The study protocol was approved by the Ethical Committee of Wroclaw Medical University (approval No. KB-794/2019). All study participants were informed of the purpose of the study and consented to participate. The study was conducted in accordance with the Declaration of Helsinki for research involving human subjects.

## Data availability

The data that supports the findings of this study is available from the corresponding author on reasonable request. The data is not publicly available due to privacy or ethical restrictions.

## Consent for publication

Not applicable.


### ORCID iDs

Sylwia Orzeszek https://orcid.org/0000-0002-3139-5696
Helena Martynowicz https://orcid.org/0000-0003-1283-8460
Joanna Smardz https://orcid.org/0000-0003-0004-3134
Anna Wojakowska https://orcid.org/0000-0002-8699-6448
Wojciech Bombała https://orcid.org/0009-0001-9989-8337
Grzegorz Mazur https://orcid.org/0000-0001-6610-2008
Mieszko Wieckiewicz https://orcid.org/0000-0003-4953-7143


### References


1. Klasser GD, Almoznino G, Fortuna G. Sleep and orofacial pain. *Dent Clin North Am*. 2018;62(4):629–656. doi:10.1016/j.cden.2018.06.005
2. Ananthan S, Benoliel R. Chronic orofacial pain. *J Neural Transm (Vienna)*. 2020;127(4):575–588. doi:10.1007/s00702-020-02157-3
3. Mier RW, Dhadwal S. Primary headaches. *Dent Clin North Am*. 2018;62(4):611–628. doi:10.1016/j.cden.2018.06.006
4. Pigg M, Nixdorf DR, Law AS, et al. New International Classification of Orofacial Pain: What is in it for endodontists? *J Endod*. doi:10.1016/j.joen.2020.12.002

5. Finan PH, Goodin BR, Smith MT. The association of sleep and pain: An update and a path forward. *J Pain*. 2013;14(12):1539–1552. doi:10.1016/j.jpain.2013.08.007
6. Dopheide JA. Insomnia overview: Epidemiology, pathophysiology, diagnosis and monitoring, and nonpharmacologic therapy. *Am J Manag Care*. 2020;26(4 Suppl):S76–S84. doi:10.37765/ajmc.2020.42769
7. Nijs J, Mairesse O, Neu D, et al. Sleep disturbances in chronic pain: Neurobiology, assessment, and treatment in physical therapist practice. *Phys Ther*. 2018;98(5):325–335. doi:10.1093/ptj/pzy020
8. Trauer JM, Qian MY, Doyle JS, Rajaratnam SMW, Cunnington D. Cognitive behavioral therapy for chronic insomnia: A systematic review and meta-analysis. *Ann Intern Med*. 2015;163(3):191–204. doi:10.7326/M14-2841
9. Haack M, Simpson N, Sethna N, Kaur S, Mullington J. Sleep deficiency and chronic pain: Potential underlying mechanisms and clinical implications. *Neuropsychopharmacology*. 2020;45(1):205–216. doi:10.1038/s41386-019-0439-z
10. Wieckiewicz M, Jenca A, Seweryn P, et al. Determination of pain intensity, pain-related disability, anxiety, depression, and perceived stress in Polish adults with temporomandibular disorders: A prospective cohort study. *Front Integr Neurosci*. 2022;16:1026781. doi:10.3389/fnint.2022.1026781
11. Wieckiewicz M, Grychowska N, Nahajowski M, et al. Prevalence and overlaps of headaches and pain-related temporomandibular disorders among the Polish urban population. *J Oral Facial Pain Headache*. 2020;34(1):31–39. doi:10.11607/ofph.2386
12. Manfredini D, Borella L, Favero L, Ferronato G, Guarda-Nardini L. Chronic pain severity and depression/somatization levels in TMD patients. *Int J Prosthodont*. 2010;23(6):529–534. PMID:21209988.
13. Manfredini D, Favero L, Del Giudice A, Masiero S, Stellini E, Guarda-Nardini L. Axis II psychosocial findings predict effectiveness of TMJ hyaluronic acid injections. *Int J Oral Maxillofac Surg*. 2013;42(3):364–368. doi:10.1016/j.ijom.2012.10.033
14. Sójka A, Stelcer B, Roy M, Mojs E, Pryliński M. Is there a relationship between psychological factors and TMD? *Brain Behav*. 2019;9(9):e01360. doi:10.1002/brb3.1360
15. Schiffman E, Ohrbach R, Truelove E, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: Recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Group†. *J Oral Facial Pain Headache*. 2014;28(1):6–27. doi:10.11607/jop.1151
16. Dreweck FDS, Soares S, Duarte J, Conti PCR, De Luca Canto G, Porporatti AL. Association between painful temporomandibular disorders and sleep quality: A systematic review. *J Oral Rehabil*. 2020;47(8):1041–1051. doi:10.1111/joor.12993
17. Almoznino G, Benoliel R, Sharav Y, Haviv Y. Sleep disorders and chronic craniofacial pain: Characteristics and management possibilities. *Sleep Med Rev*. 2017;33:39–50. doi:10.1016/j.smrv.2016.04.005
18. O'Gara BP, Gao L, Marcantonio ER, Subramaniam B. Sleep, pain, and cognition: Modifiable targets for optimal perioperative brain health. *Anesthesiology*. 2021;135(6):1132–1152. doi:10.1097/ALN.0000000000004046
19. Hudson AN, Van Dongen HPA, Honn KA. Sleep deprivation, vigilant attention, and brain function: A review. *Neuropsychopharmacology*. 2020;45(1):21–30. doi:10.1038/s41386-019-0432-6
20. Lavigne GJ, Sessle BJ. The neurobiology of orofacial pain and sleep and their interactions. *J Dent Res*. 2016;95(10):1109–1116. doi:10.1177/0022034516648264
21. Sánchez Romero EA, Martínez-Pozas O, García-González M, et al. Association between sleep disorders and sleep quality in patients with temporomandibular joint osteoarthritis: A systematic review. *Biomedicines*. 2022;10(9):2143. doi:10.3390/biomedicines10092143
22. Maślak-Bereś M, Loster JE, Wieczorek A, Loster BW. Evaluation of the psychoemotional status of young adults with symptoms of temporomandibular disorders. *Brain Behav*. 2019;9(11):e01443. doi:10.1002/brb3.1443
23. Melzack R. The short-form McGill Pain Questionnaire. *Pain*. 1987;30(2):191–197. doi:10.1016/0304-3959(87)91074-8
24. Melzack R, Katz J. The McGill Pain Questionnaire: Appraisal and current status. In: Turk DC, Melzack R, eds. *Handbook of Pain Assessment*. New York, NY: The Guilford Press; 2001:35–52.
25. Von Korff M, DeBar LL, Krebs EE, Kerns RD, Deyo RA, Keefe FJ. Graded chronic pain scale revised: Mild, bothersome, and high-impact chronic pain. *Pain*. 2020;161(3):651–661. doi:10.1097/j.pain.0000000000001758
26. Sharma S, Kallen MA, Ohrbach R. Graded Chronic Pain Scale: Validation of 1-month reference frame. *Clin J Pain*. 2021;38(2):119–131. doi:10.1097/AJP.0000000000001005
27. Yang M, Rendas-Baum R, Varon SF, Kosinski M. Validation of the Headache Impact Test (HIT-6™) across episodic and chronic migraine. *Cephalalgia*. 2011;31(3):357–367. doi:10.1177/0333102410379890
28. Stewart WF, Lipton RB, Kolodner KB, Sawyer J, Lee C, Liberman JN. Validity of the Migraine Disability Assessment (MIDAS) score in comparison to a diary-based measure in a population sample of migraine sufferers. *Pain*. 2000;88(1):41–52. doi:10.1016/S0304-3959(00)00305-5
29. Gonzalez YM, Schiffman E, Gordon SM, et al. Development of a brief and effective temporomandibular disorder pain screening questionnaire: Reliability and validity. *J Am Dent Assoc*. 2011;142(10):1183–1191. doi:10.14219/jada.archive.2011.0088
30. Bastien CH, Vallières A, Morin CM. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep Med*. 2001;2(4):297–307. doi:10.1016/s1389-9457(00)00065-4
31. Manzar MD, BaHammam AS, Hameed UA, et al. Dimensionality of the Pittsburgh Sleep Quality Index: A systematic review. *Health Qual Life Outcomes*. 2018;16(1):89. doi:10.1186/s12955-018-0915-x
32. Silva GE, Vana KD, Goodwin JL, Sherrill DL, Quan SF. Identification of patients with sleep disordered breathing: Comparing the four-variable screening tool, STOP, STOP-Bang, and Epworth Sleepiness Scales. *J Clin Sleep Med*. 2011;7(5):467–472. doi:10.5664/jcsm.1308
33. Plopa M, Makarowski R. *Kwestionariusz Poczucia Stresu*. 1st ed. Warszawa, Poland: VIZJA PRESS & IT; 2010.
34. Bastianon CD, Klein EM, Tibubos AN, Brähler E, Beutel ME, Petrowski K. Perceived Stress Scale (PSS-10) psychometric properties in migrants and native Germans. *BMC Psychiatry*. 2020;20(1):450. doi:10.1186/s12888-020-02851-2
35. Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: The PHQ primary care study. Primary Care Evaluation of Mental Disorders. Patient Health Questionnaire. *JAMA*. 1999;282(18):1737–1744. doi:10.1001/jama.282.18.1737
36. Richter P, Werner J, Heerlein A, Kraus A, Sauer H. On the validity of the Beck Depression Inventory. A review. *Psychopathology*. 1998;31(3):160–168. doi:10.1159/000066239
37. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry*. 1961;4:561–571. doi:10.1001/archpsyc.1961.01710120031004
38. Przepiorka A, Blachnio A, Cudo A. The role of depression, personality, and future time perspective in internet addiction in adolescents and emerging adults. *Psychiatry Res*. 2019;272:340–348. doi:10.1016/j.psychres.2018.12.086
39. Williams N. The GAD-7 questionnaire. *Occup Med*. 2014;64(3):224. doi:10.1093/occmed/kqt161
40. Lei J, Fu J, Yap AUJ, Fu KY. Temporomandibular disorders symptoms in Asian adolescents and their association with sleep quality and psychological distress. *Cranio*. 2016;34(4):242–249. doi:10.1179/2151090315y.0000000021
41. Florjański W, Orzeszek S. Role of mental state in temporomandibular disorders: A review of the literature. *Dent Med Probl*. 2021;58(1):127–133. doi:10.17219/dmp/132978
42. Ekici Ö. Association of stress, anxiety, and depression levels with sleep quality in patients with temporomandibular disorders. *Cranio*. 2023;41(5):407–415. doi:10.1080/08869634.2020.1861886
43. Yap AU, Cao Y, Zhang MJ, Lei J, Fu KY. Temporomandibular disorder severity and diagnostic groups: Their associations with sleep quality and impairments. *Sleep Med*. 2021;80:218–225. doi:10.1080/08869634.2019.1682750
44. Kim HK, Kim ME. Phenotyping 1488 patients with painful temporomandibular disorders and its relevance to subjective sleep quality: A key step for stratified medicine. *Cranio*. 2019;39(6):491–501. doi:10.1080/08869634.2019.1682750
45. Jiménez-Silva A, Peña-Durán C, Tobar-Reyes J, Frugone-Zambra R. Sleep and awake bruxism in adults and its relationship with temporomandibular disorders: A systematic review from 2003 to 2014. *Acta Odontol Scand*. 2017;75(1):36–58. doi:10.1080/00016357.2016.1247465

46. Bastos Machado de Resende CM, de Oliveira Medeiros FGL, de Figueiredo Rêgo CR, de Sousa Leite Bispo A, Seabra Barbosa GA, Oliveira de Almeida E. Short-term effectiveness of conservative therapies in pain, quality of life, and sleep in patients with temporomandibular disorders: A randomized clinical trial. *Cranio*. 2021;39(4):335–343. doi:10.1080/08869634.2019.1627068
47. Yatani H, Studts J, Cordova M, Carlson CR, Okeson JP. Comparison of sleep quality and clinical and psychologic characteristics in patients with temporomandibular disorders. *J Orofac Pain*. 2002;16(3):221–228. PMID:12221738.
48. Kundermann B, Hemmeter-Spernal J, Huber MT, Krieg JC, Lautenbacher S. Effects of total sleep deprivation in major depression: Overnight improvement of mood is accompanied by increased pain sensitivity and augmented pain complaints. *Psychosom Med*. 2008;70(1):92–101. doi:10.1097/PSY.0b013e31815c1b5d
49. Edwards RR, Almeida DM, Klick B, Haythornthwaite JA, Smith MT. Duration of sleep contributes to next-day pain report in the general population. *Pain*. 2008;137(1):202–207. doi:10.1016/j.pain.2008.01.025
50. Agmon M, Armon G. Increased insomnia symptoms predict the onset of back pain among employed adults. *PLoS One*. 2014;9(8):e103591. doi:10.1371/journal.pone.0103591
51. Sivertsen B, Lallukka T, Petrie KJ, Steingrímsdóttir ÓA, Stubhaug A, Nielsen CS. Sleep and pain sensitivity in adults. *Pain*. 2015;156(8):1433–1439. doi:10.1097/j.pain.0000000000000131
52. Dubrovsky B, Janal MN, Lavigne GJ, et al. Depressive symptoms account for differences between self-reported versus polysomnographic assessment of sleep quality in women with myofascial TMD. *J Oral Rehabil*. 2017;44(12):925–933. doi:10.1111/joor.12552
53. Smith MT, Wickwire EM, Grace EG, et al. Sleep disorders and their association with laboratory pain sensitivity in temporomandibular joint disorder. *Sleep*. 2009;32(6):779–790. doi:10.1093/sleep/32.6.779
54. Balbo M, Leproult R, Van Cauter E. Impact of sleep and its disturbances on hypothalamo-pituitary-adrenal axis activity. *Int J Endocrinol*. 2010:2010:759234. doi:10.1155/2010/759234
55. Herman JP, McKlveen JM, Ghosal S, et al. Regulation of the hypothalamic-pituitary-adrenocortical stress response. *Compr Physiol*. 2016;6(2):603–621. doi:10.1002/cphy.c150015

Original paper

# Dentists' knowledge about domestic violence against women: A questionnaire-based study

Suleyman Emre Meseli[1,A–F], Hulya Yildiz[2,A–D,F]

[1] Department of Periodontology, Faculty of Dentistry, Istanbul Aydin University, Turkey
[2] Istanbul Okmeydanı Oral and Dental Health Hospital, Republic of Turkey Ministry of Health, Istanbul, 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*. 2024;61(4):563–575

**Address for correspondence**
Suleyman Emre Meseli
E-mail: suleymanmeseli@aydin.edu.tr

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
The authors would like to thank M. Sc. Yusuf Iseri for the linguistic support.

Received on July 23, 2023
Reviewed on August 18, 2023
Accepted on August 24, 2023

Published online on August 26, 2024

## Abstract

**Background.** Dentists are the first healthcare professionals to identify cases of domestic violence and abuse (DVA) with head and neck injuries.

**Objectives.** The aim of this study was to assess dentists' knowledge regarding behavioral and physical findings in female victims of DVA.

**Material and methods.** The study included 558 volunteer dentists who completed a two-part questionnaire designed to assess their knowledge and awareness of DVA against women. The first part of the questionnaire inquired about the participants' demographic data, including age, sex, specialty, and the duration of professional practice. The second part assessed 15 statements on a 5-point Likert scale, concerning both behavioral (5/15) and clinical aspects (10/15) of DVA. For each statement, if a minimum of 70% of respondents selected the same option, it was interpreted as being agreed upon by the participants. The level of statistical significance was set at $p < 0.05$.

**Results.** Most of the participants were between the ages of 31 and 40 (29.1%). The study sample was predominantly female (70.4%), with 57.2% of the participants being married. The statement "Abused women tend to avoid eye contact" had the highest agreement rate (70.6%) for the behavioral assessments. However, the participants were mostly "undecided" on the remaining 4 statements in this section. In contrast, the agreement rate for 5 statements related to the clinical assessment of head, neck and intraoral injuries exceeded 70%. One-fifth of the behavioral assessment statements and half of the clinical assessment statements were negatively correlated with the age of the participants ($p < 0.05$).

**Conclusions.** The findings of this study indicate that dentists can recognize and diagnose DVA symptoms to a certain extent. However, they may encounter difficulties in identifying the suspicious behavior that is indicative of DVA.

**Keywords:** women, abuse, dentist, domestic violence, behavioral assessment

**Cite as**
Meseli SE, Yildiz H. Dentists' knowledge about domestic violence against women: A questionnaire-based study. *Dent Med Probl.* 2024;61(4):563–575. doi:10.17219/dmp/171540

**DOI**
10.17219/dmp/171540

# Introduction

The World Health Organization (WHO) defines violence as "the use of force that is likely to result in injury or loss to oneself, another person, a group, or society".[1] Although abuse is a public health problem that affects individuals, families and legal systems, it is the attitudes and behaviors of individuals that influence the physical, developmental and psychosocial well-being of others.[2] Those at risk for violence and abuse include women, children, migrants, and individuals with various forms of vulnerability and discrimination, such as those in the LGBT+ community.[3,4] Several factors have been shown to influence violent behavior, including impulsive disorders, certain mental illnesses, trauma, inadequate cultural codes, inadequate education, poverty, unemployment, and patriarchal structures.[4,5]

Women are subjected to domestic violence and abuse (DVA), especially in male-dominated societies, simply because of their gender. This social problem has existed since ancient times, from the moment that human beings first emerged into the world. According to the WHO, 35% of women globally are exposed to DVA.[2] While DVA against women has been recognized as a violation of human rights for over 30 years, it is also a manifestation of historically unequal power relations between men and women. This phenomenon is observed globally and can be observed in every country and community.[6]

Domestic violence and abuse is associated not only with physical harm but also with psychological harm.[2] Depending on the nature and extent of trauma, DVA can lead to adverse health consequences such as physical injuries, mental disorders, sexually transmitted diseases, and chronic diseases affecting various parts of the body. Additionally, cases of disability and death associated with limb loss have been reported in affected individuals.[7]

Previous studies have reported that injuries resulting from DVA occur with a frequency of 36.7% in the head and neck region.[8] Oral and maxillofacial injuries are examined under the category of head and neck injuries. The most common type of head and neck injuries is a fracture of the teeth and/or jawbones, with the incisors being the most commonly damaged teeth.[9] In addition, ecchymosis, swelling, lacerations, and rupture in the oral mucosa, temporomandibular joint problems, tooth mobility and migration, difficulty in chewing and speaking, restricted mouth opening, or tooth loss may occur.[10] In cases of DVA, dentists have the responsibility not only to examine the victim but also to report the matter to the law enforcement authorities.[11]

Based on the assumption that dentists, who have medical and legal responsibilities, are competent to distinguish DVA-subjected women, the primary aim of the study was to determine the level of dentists' knowledge about this issue.

# Material and methods

The protocol of this cross-sectional, questionnaire-based study, conducted between January 3, 2022 and March 14, 2022, was approved by the Ethics Committee for Non-interventional Research of Istanbul Aydin University, Turkey (decision No. 2021/648).

## Sample size and population

A power analysis was conducted based on the population of registered, actively practicing dentists in Turkey. The sample size was calculated based on the population of 43,199 dentists registered with the Turkish Dental Association, with a 5% margin of error and a confidence level (*CI*) between 90% and 99%. The study was to be completed with 298 dentists at a 90% *CI* or 384 dentists at a 95% *CI*. To increase the reliability of the study, 558 volunteers, registered as members of the Turkish Dental Association, were invited to participate in the study by completing a questionnaire that evaluated their knowledge and awareness of DVA against women.

All participants included in the study were required to have worked as a dentist for a minimum of 1 year, be a native Turkish speaker, be a member of the Turkish Dental Association, and volunteer to participate in the study.

## Data collection

The data collection form, which was presented to the participants, was designed as a two-part questionnaire. The first part of the questionnaire was related to the demographic characteristics of the participants, including age, sex, marital status, the duration of professional practice, specialty, the type of workplace, and the city of work. In the second part, participants were invited to complete a recently developed questionnaire about DVA against women that contained a total of 15 statements within 2 distinct subdomains, structured based on the relevant sources (Table 1).[12] This questionnaire was designed to evaluate the participants' knowledge and awareness of DVA against women. The questionnaire statements were prepared based on the relevant studies, in Turkish and English, generated by using the keywords "domestic violence," "abuse," "head and neck injuries," and "dentistry." The participants were invited to answer the questions using a 5-point Likert scale, with scores corresponding to the following statements: 1. I strongly disagree; 2. I disagree; 3. I am undecided; 4. I agree; 5. I strongly agree.

The statements evaluating the level of dentists' knowledge were developed and consulted upon with a panel consisting of an oral and maxillofacial surgeon, an oral and maxillofacial radiologist, a periodontist, a dentist, a psychiatrist, and a clinical psychologist. The questionnaire was based on Lawshe's technique.[13] The developed

Table 1. Questionnaire statements

| Category | Statement | Description |
|---|---|---|
| Behavioral assessment | St. 1 | Abused women tend to avoid eye contact. |
| | St. 2 | Abused women constantly talk about themselves and their problems. |
| | St. 3 | The facial expression of abused women is dull. |
| | St. 4 | Abuse is considered in distractible women. |
| | St. 5 | Abused women have difficulty recognizing their location, identity or time. |
| Clinical assessment | St. 6 | Injuries to the scalp in women are signs of abuse. |
| | St. 7 | The presence of bruises and injuries in the head and neck region is indicative of abuse/violence in women. |
| | St. 8 | Hard tissue injuries at the base of the patient's skull are indicative of abuse. |
| | St. 9 | The presence of hemorrhage, lacerations and ecchymotic lesions on the lips is suggestive of abuse in woman. |
| | St. 10 | The presence of subluxation in teeth is indicative of abuse in women. |
| | St. 11 | Women with a considerable number of tooth fractures are considered victims of abuse. |
| | St. 12 | Abuse is suspected in women with multiple tooth loss. |
| | St. 13 | Impairment of mucosal integrity, as well as the presence of petechial bleeding foci and rupture in soft tissues can be observed in abused women. |
| | St. 14 | The presence of simple injuries on the sublingual region is indicative of potential abuse in women. |
| | St. 15 | The presence of deep or wide lacerations, ruptures or incisions in the tongue is indicative of abuse in women. |

St – statement.

questionnaire was sent via e-mail to 5 randomly selected dentists who were not participating in the study and were employed at the Dental and Oral Health Application and Research Center of Istanbul Aydin University. After receiving the dentists' feedback, the questionnaire was revised to incorporate minor changes. Subsequently, the Google Forms link to the questionnaire was forwarded to all registered members of the Turkish Dental Association via the member e-mail system. In order to prevent bias and avoid repeated inclusion of participants in the study, one of the study authors (SEM) had access to the list of participants. The procedure was conducted in compliance with the relevant data protection regulations (Turkish Personal Data Protection Law No. 6698).

## Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics for Windows software, v. 24.0 (IBM Corp., Armonk, USA). Descriptive statistics were presented as numbers and percentages for categorical variables. Cronbach's alpha, the internal consistency test for reliability analysis, was used, and an α value of ≥0.7 and <0.8 was considered acceptable. In the case of independent groups, the ratio was compared using the $\chi^2$ test. Spearman's correlation analysis was conducted to observe the nature of the relationships between the variables. The results were analyzed with a 95% *CI* and a significance level of $p < 0.05$.

# Results

The majority of dentists who participated in the study were between 31 and 40 years of age (29.1%), while the lowest level of participation was among those aged ≥51 years (21.6%). The study sample was predominantly female (70.4%), with 57.2% of the participants being married. The majority of the participants worked in Istanbul (58.2%), and 61.7% worked in private or outpatient clinics; 38.0% of the participants have worked as dentists for 1–10 years, 31.3% had been in their profession for 11–20 years, and 60.4% had no specialty (Table 2).

The responses to the second part of the questionnaire are summarized in Table 3. The internal consistency of the questionnaire was determined with Cronbach's alpha of 0.769. With regard to statements 1 and 7 (St. 1 and St. 7), most of the respondents (70.6%) claimed that abused women avoided eye contact, while 88.9% agreed that female abuse was evident by the presence of bruises and injuries at the head and neck region (Table 3). Additionally, the presence of hard tissue lesions at the base of the patient's skull (St. 8; 88.9%), and the presence of hemorrhages, lacerations and ecchymotic lesions of the lips (St. 9; 85.4%) were identified as indicators of abuse by the majority of the participants (Table 3).

A statistically significant difference was observed in response rates for St. 7 ($p = 0.001$), St. 8 ($p = 0.032$), St. 9 ($p = 0.006$), St. 10 ($p = 0.004$), and St. 13 ($p = 0.003$) in relation to age (Table 4). With regard to the remaining

Table 2. Demographic characteristics of the participants

| Variable | | *n* | % |
|---|---|---|---|
| Age [years] | <31 | 144 | 25.9 |
| | 31–40 | 162 | 29.1 |
| | 41–50 | 131 | 23.5 |
| | >50 | 121 | 21.6 |
| Sex | male | 165 | 29.6 |
| | female | 393 | 70.4 |
| Marital status | single | 191 | 34.2 |
| | divorced/widow | 48 | 8.6 |
| | married | 319 | 57.2 |
| Duration of professional practice [years] | 1–10 | 213 | 38.0 |
| | 11–20 | 175 | 31.3 |
| | 21–30 | 73 | 13.2 |
| | >30 | 97 | 17.5 |
| Specialty | no specialty | 336 | 60.4 |
| | Endodontics | 32 | 5.9 |
| | OM Radiology | 17 | 3.0 |
| | OM Surgery | 7 | 1.1 |
| | Orthodontics | 24 | 4.0 |
| | Pediatric Dentistry | 19 | 3.5 |
| | Periodontology | 84 | 15.1 |
| | Prosthodontics | 32 | 5.9 |
| | Restorative Dentistry | 7 | 1.1 |
| Type of workplace | Ministry of Health | 108 | 19.4 |
| | Faculty of Dentistry | 106 | 18.9 |
| | private clinic | 344 | 61.7 |
| City of work | Istanbul | 326 | 58.2 |
| | Ankara | 44 | 7.8 |
| | İzmir | 50 | 8.9 |
| | others (78 of 81 cities in Turkey) | 138 | 24.9 |
| Total | | 558 | 100 |

OM – Oral & Maxillofacial.

statements, no statistically significant difference was identified with respect to the age of the participants and the duration of professional practice ($p \geq 0.05$). The proportion of "I disagree" responses was high among the participants older than 50 years for St. 7–9 and St. 13 ($p < 0.05$), while the proportion of "I am undecided" responses was high among participants below the age of 31 for St. 7–9 and St. 13 ($p < 0.05$). The proportion of "I strongly disagree" responses for St. 10 was high among participants older than 50 years, whereas the proportion of "I strongly agree" responses was high among those below the age of 31.

As shown in Table 4, a statistically significant difference was observed in response rates for St. 7 ($p = 0.020$), St. 8 ($p = 0.022$), St. 9 ($p = 0.006$), St. 10 ($p = 0.049$), and St. 13 ($p = 0.034$) in relation to the duration of professional practice. Accordingly, the proportion of "I disagree" responses among the participants who had been in the profession longer than 30 years was the highest for St. 8, St. 9 and St. 13 ($p < 0.05$). Moreover, for St. 7, St. 8 and St. 13, the proportion of "I am undecided" responses was the highest among the participants with less than 11 years of experience ($p < 0.05$). With regard to St. 10, the proportion of "I strongly disagree" responses among the participants with over 30 years of experience and the proportion of "I strongly agree" responses among the participants with less than 11 years of experience were the highest.

A comparison of response rates according to specialty revealed that only St. 4 and St. 5 of the behavioral assessment statements ($p = 0.014$ and $p = 0.024$, respectively) and all of the clinical assessment statements (except for St. 9 and St. 14) were significant ($p < 0.05$). The responses to the statements did not differ significantly with respect to sex (except for St. 7–10 and St. 13), marital status (except for St. 7), type of workplace (except for St. 13), and city of work ($p \geq 0.05$) (Table 5, 6).

Table 7 shows that St. 1, St. 7, St. 8, St. 9, St. 13, and St. 15 were negatively correlated with age ($p < 0.05$), while St. 7, St. 8, St. 9, and St. 13 demonstrated a negative association with the duration of professional practice ($p < 0.05$). All behavioral assessment statements and the remaining clinical assessment statements showed no correlation with the duration of professional practice ($p \geq 0.05$).

## Discussion

All individuals can be victims of violence, irrespective of their level of education or socioeconomic status. All instances of domestic violence represent a global health problem and are a violation of women's rights.[14] Women who are subjected to DVA exhibit anxiety or unusual behaviors during routine dental examinations and show the effects of trauma in the acute phase.[15] Domestic violence and abuse can take many forms, including neglect, as well as physical, sexual, or psychological violence. The presence of physical findings such as edema, skin bruises, fractures, cuts, burns, and scalp injuries can serve as diagnostic and investigable symptoms. As dentists do not frequently encounter cases of DVA in routine clinical practice, their ability to diagnose potential incidents of DVA is limited when patients do not disclose the cause.[16]

The analysis of the behavioral assessment responses (St. 1–5) revealed a deficiency in the participants' understanding and awareness of the psychological state of the abused women. Moreover, it is concerning that when participants encountered a woman who was distracted (St. 4) or unaware of her identity, location, or time (St. 5), they were uncertain as to whether the abuse had occurred. When a person is disoriented or confused to the point of being unaware of their own identity, any healthcare professional would be wise to assume that the individual could have been the victim of a violent or abusive act or

**Table 3.** Responses to the second part of the questionnaire

| Statement | Score | *n* | % | Cum. [%] |
|---|---|---|---|---|
| St. 1 | 1 | 12 | 2.2 | 2.2 |
| | 2 | 36 | 6.5 | 8.6 |
| | 3 | 115 | 20.8 | 29.4 |
| | 4 | 325 | 58.2 | 87.6 |
| | 5 | 70 | 12.4 | 100.0 |
| St. 2 | 1 | 42 | 7.5 | 7.5 |
| | 2 | 268 | 48.0 | 55.5 |
| | 3 | 178 | 31.8 | 87.3 |
| | 4 | 62 | 11.1 | 98.4 |
| | 5 | 8 | 1.6 | 100.0 |
| St. 3 | 1 | 59 | 10.5 | 10.5 |
| | 2 | 287 | 51.2 | 61.7 |
| | 3 | 164 | 29.4 | 91.1 |
| | 4 | 46 | 8.4 | 99.5 |
| | 5 | 2 | 0.5 | 100.0 |
| St. 4 | 1 | 16 | 2.7 | 2.7 |
| | 2 | 88 | 15.6 | 18.3 |
| | 3 | 233 | 42.0 | 60.4 |
| | 4 | 197 | 35.3 | 95.7 |
| | 5 | 24 | 4.3 | 100.0 |
| St. 5 | 1 | 12 | 2.2 | 2.2 |
| | 2 | 94 | 16.7 | 18.9 |
| | 3 | 289 | 51.8 | 70.6 |
| | 4 | 149 | 26.7 | 97.3 |
| | 5 | 14 | 2.7 | 100.0 |
| St. 6 | 1 | 2 | 0.5 | 0.5 |
| | 2 | 35 | 6.2 | 6.7 |
| | 3 | 122 | 21.8 | 28.6 |
| | 4 | 321 | 57.4 | 86.0 |
| | 5 | 78 | 14.0 | 100.0 |
| St. 7 | 1 | 5 | 0.9 | 0.9 |
| | 2 | 14 | 2.5 | 3.4 |
| | 3 | 43 | 7.7 | 11.1 |
| | 4 | 342 | 61.3 | 72.5 |
| | 5 | 154 | 27.6 | 100.0 |
| St. 8 | 1 | 2 | 0.4 | 0.4 |
| | 2 | 11 | 1.9 | 2.3 |
| | 3 | 49 | 8.8 | 11.3 |
| | 4 | 342 | 61.3 | 72.5 |
| | 5 | 154 | 27.6 | 100.0 |
| St. 9 | 1 | 1 | 0.3 | 0.3 |
| | 2 | 17 | 3.0 | 3.2 |
| | 3 | 62 | 11.3 | 14.6 |
| | 4 | 349 | 62.5 | 77.1 |
| | 5 | 129 | 22.9 | 100.0 |
| St. 10 | 1 | 6 | 1.1 | 1.1 |
| | 2 | 105 | 18.9 | 19.9 |
| | 3 | 223 | 39.9 | 59.8 |
| | 4 | 179 | 32.1 | 91.9 |
| | 5 | 45 | 8.1 | 100.0 |
| St. 11 | 1 | 6 | 1.1 | 1.1 |
| | 2 | 48 | 8.6 | 9.7 |
| | 3 | 152 | 27.2 | 36.9 |
| | 4 | 286 | 51.2 | 88.1 |
| | 5 | 66 | 11.9 | 100.0 |
| St. 12 | 1 | 17 | 3.0 | 3.0 |
| | 2 | 182 | 32.6 | 35.6 |
| | 3 | 236 | 42.3 | 77.9 |
| | 4 | 106 | 18.9 | 96.8 |
| | 5 | 17 | 3.2 | 100.0 |
| St. 13 | 1 | 6 | 1.1 | 1.1 |
| | 2 | 30 | 5.4 | 6.5 |
| | 3 | 95 | 17.0 | 23.5 |
| | 4 | 344 | 61.7 | 85.2 |
| | 5 | 83 | 14.8 | 100.0 |
| St. 14 | 1 | 7 | 1.3 | 1.3 |
| | 2 | 90 | 16.2 | 17.5 |
| | 3 | 179 | 32.1 | 49.6 |
| | 4 | 266 | 47.7 | 97.3 |
| | 5 | 16 | 2.7 | 100.0 |
| St. 15 | 1 | 2 | 0.5 | 0.5 |
| | 2 | 46 | 8.1 | 8.6 |
| | 3 | 155 | 27.8 | 36.4 |
| | 4 | 278 | 49.9 | 86.3 |
| | 5 | 77 | 13.7 | 100.0 |

Cum. – cumulative.

may require urgent medical attention. Although the psychological evaluation of the DVA victim is not the primary responsibility of the dentist, it is important to recognize that behavioral findings can shed light on clinical findings.

A recent study by de Macedo Bernardino et al. demonstrated that physical assault against women was more prevalent than other forms of DVA (85.2%), and that women were more likely to be abused in their own homes (74.2%).[17] The authors found that 45.8% of cases exhibited trauma to the oral and maxillofacial regions, characterized by injuries. Other studies have reported that trauma to the oropharyngeal area was associated with other instances of violent acts that affected an individual's quality of life, resulting in scarring, difficulty in mastication, or a tendency to avoid close relationships with other men.[18]

**Table 4.** Comparison of the responses to the second part of the questionnaire regarding age and the duration of professional practice

| Statement | Score | Age [years] | | | | | Duration of professional practice [years] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | <31 | 31–40 | 41–50 | >50 | *p*-value | 1–10 | 11–20 | 21–30 | >30 | *p*-value |
| St. 1 | 1 | 3.1 | 1.9 | 1.1 | 2.5 | 0.341 | 3.5 | 0.0 | 2.0 | 3.1 | 0.403 |
| | 2 | 1.0 | 8.3 | 9.2 | 7.5 | | 6.4 | 6.0 | 6.1 | 7.7 | |
| | 3 | 16.7 | 19.4 | 23.0 | 25.0 | | 14.9 | 23.3 | 28.6 | 23.1 | |
| | 4 | 65.6 | 55.6 | 55.2 | 56.3 | | 61.7 | 56.0 | 57.1 | 55.4 | |
| | 5 | 13.5 | 14.8 | 11.5 | 8.8 | | 13.5 | 14.7 | 6.1 | 10.8 | |
| St. 2 | 1 | 9.4 | 6.5 | 6.9 | 7.5 | 0.368 | 9.2 | 5.2 | 6.1 | 9.2 | 0.520 |
| | 2 | 49.0 | 48.1 | 55.2 | 38.8 | | 48.2 | 50.0 | 46.9 | 44.6 | |
| | 3 | 33.3 | 32.4 | 21.8 | 40.0 | | 30.5 | 32.8 | 24.5 | 38.5 | |
| | 4 | 8.3 | 12.0 | 12.6 | 11.3 | | 11.3 | 9.5 | 20.4 | 6.2 | |
| | 5 | 0.0 | 0.9 | 3.4 | 2.5 | | 0.7 | 2.6 | 2.0 | 1.5 | |
| St. 3 | 1 | 12.5 | 11.1 | 8.0 | 10.0 | 0.374 | 11.3 | 12.1 | 4.1 | 10.8 | 0.228 |
| | 2 | 50.0 | 50.0 | 62.1 | 42.5 | | 48.9 | 54.3 | 61.2 | 43.1 | |
| | 3 | 30.2 | 30.6 | 25.3 | 31.3 | | 33.3 | 25.9 | 28.6 | 27.7 | |
| | 4 | 7.3 | 7.4 | 4.6 | 15.0 | | 6.4 | 6.9 | 6.1 | 16.9 | |
| | 5 | 0.0 | 0.9 | 0.0 | 1.3 | | 0.0 | 0.9 | 0.0 | 1.5 | |
| St. 4 | 1 | 0.0 | 2.8 | 2.3 | 6.3 | 0.636 | 1.4 | 1.7 | 2.0 | 7.7 | 0.581 |
| | 2 | 15.6 | 12.0 | 18.4 | 17.5 | | 16.3 | 13.8 | 16.3 | 16.9 | |
| | 3 | 45.8 | 44.4 | 40.2 | 36.3 | | 44.7 | 44.0 | 38.8 | 35.4 | |
| | 4 | 34.4 | 37.0 | 33.3 | 36.3 | | 33.3 | 37.9 | 34.7 | 35.4 | |
| | 5 | 4.2 | 1.9 | 1.1 | 2.5 | | 4.3 | 2.6 | 8.2 | 4.6 | |
| St. 5 | 1 | 3.1 | 1.9 | 0.0 | 3.8 | 0.499 | 3.5 | 0.0 | 0.0 | 4.6 | 0.428 |
| | 2 | 12.5 | 13.9 | 19.5 | 22.5 | | 14.2 | 15.5 | 22.4 | 20.0 | |
| | 3 | 55.2 | 53.7 | 51.7 | 45.0 | | 53.2 | 54.3 | 44.9 | 49.2 | |
| | 4 | 24.0 | 28.7 | 26.4 | 27.5 | | 25.5 | 26.7 | 32.7 | 24.6 | |
| | 5 | 5.2 | 1.9 | 2.3 | 1.3 | | 3.5 | 3.4 | 0.0 | 1.5 | |
| St. 6 | 1 | 0.0 | 0.9 | 0.0 | 1.3 | 0.239 | 0.7 | 0.0 | 0.0 | 1.5 | 0.199 |
| | 2 | 3.1 | 5.6 | 3.4 | 13.8 | | 5.0 | 4.3 | 6.1 | 12.3 | |
| | 3 | 22.9 | 21.3 | 25.3 | 17.5 | | 22.0 | 27.6 | 14.3 | 16.9 | |
| | 4 | 59.4 | 55.6 | 56.3 | 58.8 | | 57.4 | 50.9 | 69.4 | 60.0 | |
| | 5 | 14.6 | 16.7 | 14.9 | 8.8 | | 14.9 | 17.2 | 10.2 | 9.2 | |
| St. 7 | 1 | 4.2 | 0.0 | 0.0 | 3.8 | 0.001* | 0.0 | 0.0 | 0.0 | 1.5 | 0.020* |
| | 2 | 0.0 | 2.8 | 3.4 | 5.0 | | 2.8 | 1.7 | 6.1 | 4.6 | |
| | 3 | 33.3 | 11.1 | 2.3 | 10.0 | | 27.8 | 8.6 | 4.1 | 9.2 | |
| | 4 | 7.3 | 54.6 | 73.6 | 63.8 | | 32.5 | 66.4 | 69.4 | 64.6 | |
| | 5 | 55.2 | 31.5 | 20.7 | 17.5 | | 36.9 | 23.3 | 20.4 | 20.0 | |
| St. 8 | 1 | 1.0 | 0.0 | 0.0 | 2.5 | 0.032* | 0.0 | 0.0 | 0.0 | 0.0 | 0.022* |
| | 2 | 0.0 | 1.9 | 2.3 | 2.5 | | 2.1 | 2.6 | 2.0 | 3.1 | |
| | 3 | 37.5 | 8.3 | 8.0 | 13.8 | | 27.8 | 7.8 | 6.1 | 15.4 | |
| | 4 | 6.3 | 57.4 | 69.0 | 65.0 | | 33.2 | 67.2 | 65.3 | 64.6 | |
| | 5 | 55.2 | 32.4 | 20.7 | 16.3 | | 36.9 | 22.4 | 26.5 | 16.9 | |
| St. 9 | 1 | 1.0 | 0.0 | 0.0 | 4.3 | 0.006* | 0.0 | 0.0 | 0.0 | 1.5 | 0.006* |
| | 2 | 2.0 | 2.8 | 2.3 | 6.3 | | 2.8 | 1.7 | 1.1 | 3.1 | |
| | 3 | 25.3 | 13.0 | 12.6 | 11.3 | | 8.5 | 17.2 | 6.1 | 10.8 | |
| | 4 | 40.4 | 52.8 | 71.3 | 68.8 | | 58.9 | 58.6 | 73.5 | 69.2 | |
| | 5 | 30.2 | 31.5 | 13.8 | 12.5 | | 29.8 | 22.4 | 19.3 | 15.4 | |

| Statement | Score | Age [years] | | | | | Duration of professional practice [years] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | <31 | 31–40 | 41–50 | >50 | *p*-value | 1–10 | 11–20 | 21–30 | >30 | *p*-value |
| St. 10 | 1 | 0.0 | 0.9 | 0.0 | 3.8 | 0.004* | 0.0 | 0.9 | 2.0 | 3.1 | 0.049* |
| | 2 | 13.5 | 16.7 | 25.3 | 21.3 | | 15.6 | 20.7 | 18.4 | 20.1 | |
| | 3 | 31.0 | 36.1 | 32.2 | 40.0 | | 44.7 | 41.2 | 42.7 | 44.5 | |
| | 4 | 28.1 | 30.6 | 36.8 | 33.8 | | 29.1 | 32.8 | 30.8 | 30.8 | |
| | 5 | 27.3 | 15.7 | 5.7 | 1.3 | | 10.6 | 5.5 | 6.1 | 1.5 | |
| St. 11 | 1 | 0.0 | 0.9 | 2.3 | 1.3 | 0.123 | 0.0 | 1.7 | 2.0 | 1.5 | 0.173 |
| | 2 | 6.3 | 8.3 | 11.5 | 8.8 | | 7.1 | 11.2 | 10.2 | 6.2 | |
| | 3 | 32.3 | 21.3 | 26.4 | 30.0 | | 30.5 | 21.6 | 22.4 | 33.8 | |
| | 4 | 47.9 | 50.9 | 50.6 | 56.3 | | 47.5 | 51.7 | 57.1 | 53.8 | |
| | 5 | 13.5 | 18.5 | 9.2 | 3.8 | | 14.9 | 13.8 | 8.2 | 4.6 | |
| St. 12 | 1 | 2.1 | 3.7 | 2.3 | 3.8 | 0.228 | 3.5 | 1.7 | 2.0 | 4.6 | 0.468 |
| | 2 | 30.2 | 29.6 | 39.1 | 32.5 | | 29.8 | 37.9 | 28.6 | 32.3 | |
| | 3 | 46.9 | 40.7 | 43.7 | 37.5 | | 46.1 | 37.9 | 49.0 | 36.9 | |
| | 4 | 18.8 | 20.4 | 10.3 | 26.3 | | 17.7 | 18.1 | 14.3 | 26.2 | |
| | 5 | 2.1 | 5.6 | 4.6 | 0.0 | | 2.8 | 4.3 | 6.1 | 0.0 | |
| St. 13 | 1 | 1.0 | 0.9 | 0.0 | 2.5 | 0.003* | 1.4 | 7.0 | 5.9 | 3.1 | 0.034* |
| | 2 | 3.1 | 4.6 | 9.2 | 15.0 | | 0.3 | 0.8 | 0.3 | 3.1 | |
| | 3 | 32.5 | 16.7 | 17.2 | 12.5 | | 36.8 | 20.7 | 16.3 | 20.0 | |
| | 4 | 20.4 | 56.5 | 65.5 | 66.3 | | 41.0 | 55.2 | 69.4 | 69.2 | |
| | 5 | 42.9 | 21.3 | 8.0 | 3.8 | | 20.6 | 16.4 | 8.2 | 4.6 | |
| St. 14 | 1 | 1.0 | 2.8 | 1.1 | 0.0 | 0.751 | 2.1 | 1.7 | 0.0 | 0.0 | 0.860 |
| | 2 | 16.7 | 17.6 | 13.8 | 16.3 | | 15.6 | 19.8 | 10.2 | 15.4 | |
| | 3 | 30.2 | 35.2 | 28.7 | 33.8 | | 32.6 | 31.0 | 32.7 | 32.3 | |
| | 4 | 49.0 | 40.7 | 52.9 | 50.0 | | 46.8 | 44.0 | 53.1 | 52.3 | |
| | 5 | 3.1 | 3.7 | 3.4 | 0.0 | | 2.8 | 3.4 | 4.1 | 0.0 | |
| St. 15 | 1 | 0.0 | 0.9 | 0.0 | 1.3 | 0.518 | 0.7 | 0.0 | 0.0 | 1.5 | 0.737 |
| | 2 | 6.3 | 7.4 | 8.0 | 11.3 | | 7.8 | 7.8 | 6.1 | 10.8 | |
| | 3 | 22.9 | 26.9 | 35.6 | 26.3 | | 24.1 | 33.6 | 26.5 | 26.2 | |
| | 4 | 52.1 | 49.1 | 47.1 | 51.3 | | 49.6 | 46.6 | 55.1 | 52.3 | |
| | 5 | 18.8 | 15.7 | 9.2 | 10.0 | | 17.7 | 12.1 | 12.2 | 9.2 | |

* statistically significant ($p < 0.05$, $\chi^2$ test). Data presented as percentage (%).

A study conducted in South Asia revealed that 50.4% of women who were victims of DVA had frequent bruises, abrasions and lacerations of the jaw.[19] Although facial and soft tissue injuries are typically considered serious, they can have aesthetic and functional consequences for women who are victims of DVA.[20] A study conducted in 22,822 Turkish households (88% response rate) with women aged 15 to 59 years found that 39% of the women had experienced physical DVA.[21] According to the results of the 2015 survey on domestic violence against women in Turkey, 36% of women had been subjected to physical DVA.[22] The results of the abovementioned studies demonstrate that DVA remains a serious problem in developing countries.[19–22]

The responses given in this study to the statements about the clinical findings show the competence of dentists in recognizing cases of DVA. There was a substantial consensus among participants in response to statements regarding the extraoral areas of the head and neck trauma (St. 6–8), indicating a high level of familiarity with the injured body region. There was a strong agreement among participants for St. 9 and St. 13, which pertained to clinical assessments of both the intraoral and perioral areas. A consensus was reached for St. 11 and St. 15, suggesting that these statements were accepted by the majority of the participants (≥60%). On the other hand, the rate of undecided respondents, which exceeded 25% for both statements, should be taken into account when

**Table 5.** Comparison of the responses to the second part of the questionnaire regarding sex, specialty and marital status

| Statement | Score | Sex | | | Specialty | | | Marital status | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M | F | *p*-value | yes | no | *p*-value | S | D/W | M | *p*-value |
| St. 1 | 1 | 3.6 | 1.5 | 0.403 | 1.3 | 3.4 | 0.556 | 4.7 | 0.0 | 0.9 | 0.076 |
| | 2 | 7.3 | 6.1 | | 6.7 | 6.1 | | 3.9 | 15.6 | 6.6 | |
| | 3 | 23.6 | 19.5 | | 22.8 | 17.7 | | 17.3 | 12.5 | 24.1 | |
| | 4 | 54.5 | 59.8 | | 56.7 | 60.5 | | 59.8 | 56.3 | 57.5 | |
| | 5 | 10.9 | 13.0 | | 12.5 | 12.2 | | 14.2 | 15.6 | 10.8 | |
| St. 2 | 1 | 11.8 | 5.7 | 0.520 | 9.4 | 4.8 | 0.190 | 6.3 | 6.3 | 8.5 | 0.240 |
| | 2 | 42.7 | 50.2 | | 44.2 | 53.7 | | 54.3 | 50.0 | 43.9 | |
| | 3 | 30.9 | 32.2 | | 33.5 | 29.3 | | 29.9 | 34.4 | 32.5 | |
| | 4 | 11.8 | 10.7 | | 12.1 | 9.5 | | 8.7 | 3.1 | 13.7 | |
| | 5 | 2.7 | 1.1 | | 0.9 | 2.7 | | 0.8 | 6.3 | 1.4 | |
| St. 3 | 1 | 7.3 | 11.9 | 0.228 | 8.9 | 12.9 | 0.421 | 13.4 | 3.1 | 9.9 | 0.308 |
| | 2 | 54.5 | 49.8 | | 52.2 | 49.7 | | 54.3 | 50.0 | 49.5 | |
| | 3 | 27.3 | 30.3 | | 29.5 | 29.3 | | 24.4 | 31.3 | 32.1 | |
| | 4 | 10.9 | 7.3 | | 8.9 | 7.5 | | 7.9 | 12.5 | 8.0 | |
| | 5 | 0.0 | 0.8 | | 0.4 | 0.7 | | 0.0 | 3.1 | 0.5 | |
| St. 4 | 1 | 5.5 | 1.5 | 0.581 | 3.1 | 2.0 | 0.014* | 1.6 | 6.3 | 2.8 | 0.798 |
| | 2 | 21.8 | 13.0 | | 19.6 | 9.5 | | 16.5 | 18.8 | 14.6 | |
| | 3 | 43.6 | 41.4 | | 39.3 | 46.3 | | 44.9 | 34.4 | 41.5 | |
| | 4 | 25.5 | 39.5 | | 34.4 | 36.7 | | 33.1 | 34.4 | 36.8 | |
| | 5 | 3.6 | 4.6 | | 3.6 | 5.4 | | 3.9 | 6.3 | 4.2 | |
| St. 5 | 1 | 4.5 | 1.1 | 0.428 | 2.2 | 2.0 | 0.024* | 2.4 | 3.1 | 1.9 | 0.974 |
| | 2 | 23.6 | 13.8 | | 21.0 | 10.2 | | 15.0 | 15.6 | 17.9 | |
| | 3 | 44.5 | 54.8 | | 46.4 | 59.9 | | 52.8 | 46.9 | 51.9 | |
| | 4 | 23.6 | 28.0 | | 27.2 | 25.9 | | 26.8 | 31.3 | 25.9 | |
| | 5 | 3.6 | 2.3 | | 3.1 | 2.0 | | 3.1 | 3.1 | 2.4 | |
| St. 6 | 1 | 0.9 | 0.4 | 0.199 | 0.4 | 0.7 | 0.033* | 0.0 | 0.0 | 0.9 | 0.585 |
| | 2 | 11.8 | 3.8 | | 7.6 | 4.1 | | 6.3 | 12.5 | 5.2 | |
| | 3 | 19.1 | 23.0 | | 20.1 | 24.5 | | 18.1 | 28.1 | 23.1 | |
| | 4 | 51.8 | 59.8 | | 59.4 | 54.4 | | 61.4 | 16 | 56.1 | |
| | 5 | 16.4 | 13.0 | | 12.5 | 16.3 | | 14.2 | 3 | 14.6 | |
| St. 7 | 1 | 2.7 | 0.0 | 0.020* | 0.9 | 0.7 | 0.001* | 0.0 | 0.0 | 1.4 | 0.026* |
| | 2 | 5.5 | 1.5 | | 3.6 | 1.4 | | 0.8 | 3.1 | 3.8 | |
| | 3 | 11.8 | 6.1 | | 8.0 | 7.5 | | 7.1 | 12.5 | 7.5 | |
| | 4 | 63.6 | 60.2 | | 63.8 | 57.1 | | 53.5 | 65.6 | 65.1 | |
| | 5 | 16.4 | 32.2 | | 23.7 | 33.3 | | 38.6 | 18.8 | 22.2 | |
| St. 8 | 1 | 1.8 | 0.0 | 0.022* | 0.4 | 0.7 | 0.001* | 0.0 | 0.0 | 0.9 | 0.179 |
| | 2 | 4.5 | 0.8 | | 2.2 | 1.4 | | 1.6 | 3.1 | 1.9 | |
| | 3 | 13.6 | 6.9 | | 9.4 | 8.2 | | 5.5 | 15.6 | 9.9 | |
| | 4 | 60.9 | 61.3 | | 63.4 | 57.8 | | 57.5 | 59.4 | 63.7 | |
| | 5 | 19.1 | 31.0 | | 24.6 | 32.0 | | 35.4 | 21.9 | 23.6 | |
| St. 9 | 1 | 0.9 | 0.0 | 0.006* | 0.4 | 0.0 | 0.060 | 0.0 | 0.0 | 0.5 | 0.381 |
| | 2 | 4.5 | 2.3 | | 4.0 | 1.4 | | 1.6 | 3.1 | 3.8 | |
| | 3 | 13.6 | 10.3 | | 12.9 | 8.8 | | 13.4 | 12.5 | 9.9 | |
| | 4 | 65.5 | 61.3 | | 61.6 | 63.9 | | 55.9 | 65.6 | 66.0 | |
| | 5 | 15.5 | 26.1 | | 21.0 | 25.9 | | 29.1 | 18.8 | 19.8 | |

| Statement | Score | Sex | | | Specialty | | | Marital status | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M | F | *p*-value | yes | no | *p*-value | S | D/W | M | *p*-value |
| St. 10 | 1 | 3.6 | 0.0 | 0.049* | 1.3 | 0.7 | 0.005* | 0.0 | 0.0 | 1.9 | 0.130 |
| | 2 | 24.5 | 16.5 | | 21.9 | 14.3 | | 15.7 | 28.1 | 19.3 | |
| | 3 | 40.9 | 39.5 | | 39.7 | 40.1 | | 48.0 | 46.9 | 34.0 | |
| | 4 | 25.5 | 34.9 | | 29.0 | 36.7 | | 29.1 | 21.9 | 35.4 | |
| | 5 | 5.5 | 9.2 | | 8.0 | 8.2 | | 7.1 | 3.1 | 9.4 | |
| St. 11 | 1 | 2.7 | 0.4 | 0.173 | 1.3 | 0.7 | 0.001* | 0.8 | 0.0 | 1.4 | 0.129 |
| | 2 | 17.3 | 5.0 | | 9.4 | 7.5 | | 7.1 | 12.5 | 9.0 | |
| | 3 | 31.8 | 25.3 | | 29.0 | 24.5 | | 34.6 | 37.5 | 21.2 | |
| | 4 | 37.3 | 57.1 | | 50.0 | 53.1 | | 46.5 | 46.9 | 54.7 | |
| | 5 | 10.9 | 12.3 | | 10.3 | 14.3 | | 11.0 | 3.1 | 13.7 | |
| St. 12 | 1 | 7.3 | 1.1 | 0.468 | 3.6 | 2.0 | 0.003* | 3.1 | 3.1 | 2.8 | 0.602 |
| | 2 | 40.9 | 29.1 | | 34.4 | 29.9 | | 29.1 | 37.5 | 34.0 | |
| | 3 | 33.6 | 46.0 | | 37.9 | 49.0 | | 47.2 | 46.9 | 38.7 | |
| | 4 | 14.5 | 20.7 | | 20.5 | 16.3 | | 18.9 | 9.4 | 20.3 | |
| | 5 | 3.6 | 3.1 | | 3.6 | 2.7 | | 1.6 | 3.1 | 4.2 | |
| St. 13 | 1 | 2.7 | 0.4 | 0.034* | 0.9 | 1.4 | 0.001* | 0.8 | 0.0 | 1.4 | 0.548 |
| | 2 | 10.0 | 3.4 | | 7.1 | 2.7 | | 6.3 | 6.3 | 4.7 | |
| | 3 | 24.5 | 13.8 | | 19.6 | 12.9 | | 13.4 | 18.8 | 18.9 | |
| | 4 | 52.7 | 65.5 | | 60.3 | 63.9 | | 59.8 | 68.8 | 61.8 | |
| | 5 | 10.0 | 16.9 | | 12.1 | 19.0 | | 19.7 | 6.3 | 13.2 | |
| St. 14 | 1 | 0.9 | 1.5 | 0.860 | 1.3 | 1.4 | 0.499 | 1.6 | 0.0 | 1.4 | 0.367 |
| | 2 | 20.9 | 14.2 | | 14.3 | 19.0 | | 18.1 | 3.1 | 17.0 | |
| | 3 | 28.2 | 33.7 | | 32.1 | 32.0 | | 29.1 | 43.8 | 32.1 | |
| | 4 | 48.2 | 47.5 | | 48.7 | 46.3 | | 48.8 | 46.9 | 47.2 | |
| | 5 | 1.8 | 3.1 | | 3.6 | 1.4 | | 2.4 | 6.3 | 2.4 | |
| St. 15 | 1 | 0.9 | 0.4 | 0.737 | 0.4 | 0.7 | 0.010* | 0.0 | 0.0 | 0.9 | 0.577 |
| | 2 | 13.6 | 5.7 | | 7.6 | 8.8 | | 9.4 | 12.5 | 6.6 | |
| | 3 | 33.6 | 25.3 | | 31.3 | 22.4 | | 26.0 | 21.9 | 29.7 | |
| | 4 | 38.2 | 54.8 | | 46.9 | 54.4 | | 48.0 | 46.9 | 51.4 | |
| | 5 | 13.6 | 13.8 | | 13.8 | 13.6 | | 16.5 | 18.8 | 11.3 | |

* statistically significant ($p < 0.05$, $\chi^2$ test). Data presented as percentage (%). M – male; F – female; S – single; D/W – divorced/widow; M – married.

interpreting the outcomes. With regard to the clinical assessment statements, half of the participants agreed upon St. 14. However, more than one-third of the participants were undecided and could not make a clear assessment of the case described in this statement. The majority of the participants disagreed or were undecided on their response regarding St. 12. Therefore, it was assumed that either the content of St. 12 was not clearly defined to the participants or that the participants responded to this statement with connotations of some clinical conditions other than those described in St. 12. The findings of this study indicate that dentists are capable of detecting physical symptoms in women who are likely to be victims of DVA. However, even professionals with high sociocultural and educational qualifications may encounter difficulties in recognizing behavioral changes associated with DVA.

Many clinicians refer to the process of screening for DVA as "opening Pandora's box".[23] Clinicians state that the observation of the victim's behavior is a key factor in diagnosing the presence of abuse,[24] but many medical professionals believe this is not common among their patients.[25,26] In some studies, the majority of women indicated that they would prefer medical professionals to ask them directly about the abuse or to respond to the victim's signs.[27] The majority of dentists (87%) reported that they had never treated such patients, 18% stated that they had never encountered a patient with apparent head and neck trauma, and 23% indicated that cases of DVA were not within their scope of practice.[28]

**Table 6.** Comparison of the responses to the second part of the questionnaire with regard to the type of workplace and the city of work

| Statement | Score | Type of workplace | | | | City of work | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Faculty of Dentistry | private practice | Ministry of Health | *p*-value | Ankara | Istanbul | İzmir | others | *p*-value |
| St. 1 | 1 | 1.4 | 3.1 | 0.0 | 0.800 | 0.0 | 2.8 | 0.0 | 2.2 | 0.884 |
| | 2 | 5.7 | 7.0 | 5.6 | | 3.4 | 6.9 | 0.0 | 8.7 | |
| | 3 | 20.0 | 20.1 | 23.6 | | 27.6 | 19.9 | 21.2 | 20.7 | |
| | 4 | 57.1 | 59.4 | 55.6 | | 62.1 | 58.3 | 66.7 | 53.3 | |
| | 5 | 15.7 | 10.5 | 15.3 | | 6.9 | 12.0 | 12.1 | 15.2 | |
| St. 2 | 1 | 2.9 | 9.2 | 6.9 | 0.246 | 13.8 | 5.6 | 12.1 | 8.7 | 0.086 |
| | 2 | 51.4 | 49.3 | 40.3 | | 55.2 | 49.1 | 57.6 | 39.1 | |
| | 3 | 32.9 | 30.1 | 36.1 | | 20.7 | 32.4 | 27.3 | 35.9 | |
| | 4 | 8.6 | 10.5 | 15.3 | | 6.9 | 12.5 | 3.0 | 12.0 | |
| | 5 | 4.3 | 0.9 | 1.4 | | 3.4 | 0.5 | 0.0 | 4.3 | |
| St. 3 | 1 | 15.7 | 8.3 | 12.5 | 0.229 | 3.4 | 12.5 | 3.0 | 10.9 | 0.660 |
| | 2 | 42.9 | 52.4 | 55.6 | | 58.6 | 52.3 | 57.6 | 44.6 | |
| | 3 | 34.3 | 30.6 | 20.8 | | 34.5 | 27.3 | 30.3 | 32.6 | |
| | 4 | 5.7 | 8.3 | 11.1 | | 3.4 | 7.4 | 9.1 | 10.9 | |
| | 5 | 1.4 | 0.4 | 0.0 | | 0.0 | 0.5 | 0.0 | 1.1 | |
| St. 4 | 1 | 1.4 | 3.5 | 1.4 | 0.146 | 0.0 | 2.8 | 3.0 | 3.3 | 0.830 |
| | 2 | 8.6 | 20.1 | 8.3 | | 13.8 | 15.3 | 27.3 | 13.0 | |
| | 3 | 50.0 | 37.6 | 48.6 | | 62.1 | 39.4 | 42.4 | 42.4 | |
| | 4 | 35.7 | 34.5 | 37.5 | | 24.1 | 37.0 | 27.3 | 38.0 | |
| | 5 | 4.3 | 4.4 | 4.2 | | 0.0 | 5.6 | 0.0 | 3.3 | |
| St. 5 | 1 | 1.4 | 2.6 | 1.4 | 0.387 | 3.4 | 0.9 | 3.0 | 4.3 | 0.137 |
| | 2 | 10.0 | 19.2 | 15.3 | | 3.4 | 17.6 | 24.2 | 15.2 | |
| | 3 | 58.6 | 52.0 | 44.4 | | 75.9 | 51.4 | 54.5 | 44.6 | |
| | 4 | 28.6 | 23.6 | 34.7 | | 17.2 | 27.3 | 18.2 | 31.5 | |
| | 5 | 1.4 | 2.6 | 4.2 | | 0.0 | 2.8 | 0.0 | 4.3 | |
| St. 6 | 1 | 0.0 | 0.9 | 0.0 | 0.988 | 3.4 | 0.0 | 3.0 | 0.0 | 0.649 |
| | 2 | 5.7 | 6.6 | 5.6 | | 3.4 | 5.6 | 3.0 | 9.8 | |
| | 3 | 20.0 | 21.8 | 23.6 | | 27.6 | 21.3 | 27.3 | 19.6 | |
| | 4 | 58.6 | 58.1 | 54.2 | | 55.2 | 61.1 | 54.5 | 50.0 | |
| | 5 | 15.7 | 12.7 | 16.7 | | 10.3 | 12.0 | 12.1 | 20.7 | |
| St. 7 | 1 | 0.0 | 1.3 | 0.0 | 0.893 | 0.0 | 0.5 | 3.0 | 1.1 | 0.473 |
| | 2 | 1.4 | 3.5 | 1.4 | | 0.0 | 2.8 | 3.0 | 3.3 | |
| | 3 | 7.1 | 7.4 | 9.7 | | 6.9 | 9.3 | 9.1 | 4.3 | |
| | 4 | 58.6 | 60.7 | 65.3 | | 72.4 | 57.9 | 66.7 | 63.0 | |
| | 5 | 32.9 | 27.1 | 23.6 | | 20.7 | 29.6 | 18.2 | 28.3 | |
| St. 8 | 1 | 0.0 | 0.9 | 0.0 | 0.695 | 0.0 | 0.0 | 3.0 | 1.1 | 0.119 |
| | 2 | 1.4 | 1.7 | 2.8 | | 0.0 | 1.4 | 0.0 | 4.3 | |
| | 3 | 8.6 | 7.9 | 12.5 | | 10.3 | 6.5 | 18.2 | 10.9 | |
| | 4 | 58.6 | 60.7 | 65.3 | | 65.5 | 62.5 | 63.6 | 56.5 | |
| | 5 | 31.4 | 28.8 | 19.4 | | 24.1 | 29.6 | 15.2 | 27.2 | |
| St. 9 | 1 | 0.0 | 0.4 | 0.0 | 0.228 | 0.0 | 0.0 | 3.0 | 0.0 | 0.192 |
| | 2 | 1.4 | 3.9 | 1.4 | | 0.0 | 2.3 | 3.0 | 5.4 | |
| | 3 | 7.1 | 10.5 | 18.1 | | 20.7 | 8.3 | 12.1 | 15.2 | |
| | 4 | 64.3 | 60.7 | 66.7 | | 55.2 | 65.3 | 63.6 | 57.6 | |
| | 5 | 27.1 | 24.5 | 13.9 | | 24.1 | 24.1 | 18.2 | 21.7 | |

| Statement | Score | Type of workplace | | | | City of work | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Faculty of Dentistry | private practice | Ministry of Health | *p*-value | Ankara | Istanbul | İzmir | others | *p*-value |
| St. 10 | 1 | 0.0 | 1.7 | 0.0 | 0.259 | 0.0 | 1.4 | 3.0 | 0.0 | 0.491 |
| | 2 | 14.3 | 17.9 | 26.4 | | 6.9 | 17.1 | 24.2 | 25.0 | |
| | 3 | 48.6 | 39.7 | 31.9 | | 41.4 | 40.7 | 42.4 | 37.0 | |
| | 4 | 27.1 | 31.9 | 37.5 | | 41.4 | 32.9 | 27.3 | 28.3 | |
| | 5 | 10.0 | 8.7 | 4.2 | | 10.3 | 7.9 | 3.0 | 9.8 | |
| St. 11 | 1 | 0.0 | 1.7 | 0.0 | 0.424 | 0.0 | 0.9 | 3.0 | 1.1 | 0.484 |
| | 2 | 5.7 | 7.4 | 15.3 | | 3.4 | 6.5 | 12.1 | 14.1 | |
| | 3 | 27.1 | 27.5 | 26.4 | | 27.6 | 27.3 | 30.3 | 25.0 | |
| | 4 | 50.0 | 52.4 | 48.6 | | 55.2 | 52.8 | 51.5 | 46.7 | |
| | 5 | 17.1 | 10.9 | 9.7 | | 13.8 | 12.5 | 3.0 | 13.0 | |
| St. 12 | 1 | 2.9 | 3.1 | 2.8 | 0.937 | 6.9 | 2.8 | 3.0 | 2.2 | 0.764 |
| | 2 | 28.6 | 31.4 | 40.3 | | 20.7 | 35.2 | 30.3 | 30.4 | |
| | 3 | 44.3 | 42.8 | 38.9 | | 51.7 | 38.9 | 42.4 | 47.8 | |
| | 4 | 20.0 | 19.7 | 15.3 | | 17.2 | 20.4 | 21.2 | 15.2 | |
| | 5 | 4.3 | 3.1 | 2.8 | | 3.4 | 2.8 | 3.0 | 4.3 | |
| St. 13 | 1 | 0.0 | 1.7 | 0.0 | 0.002* | 3.4 | 0.9 | 3.0 | 0.0 | 0.617 |
| | 2 | 4.3 | 5.2 | 6.9 | | 6.9 | 4.2 | 3.0 | 8.7 | |
| | 3 | 14.3 | 18.3 | 15.3 | | 13.8 | 16.2 | 15.2 | 20.7 | |
| | 4 | 55.7 | 59.0 | 76.4 | | 58.6 | 63.9 | 60.6 | 57.6 | |
| | 5 | 25.7 | 15.7 | 1.4 | | 17.2 | 14.8 | 18.2 | 13.0 | |
| St. 14 | 1 | 1.4 | 1.7 | 0.0 | 0.709 | 3.4 | 1.4 | 0.0 | 1.1 | 0.871 |
| | 2 | 22.9 | 14.0 | 16.7 | | 10.3 | 17.1 | 12.1 | 17.4 | |
| | 3 | 27.1 | 34.1 | 30.6 | | 41.4 | 29.2 | 36.4 | 34.8 | |
| | 4 | 47.1 | 47.6 | 48.6 | | 41.4 | 49.1 | 48.5 | 45.7 | |
| | 5 | 1.4 | 2.6 | 4.2 | | 3.4 | 3.2 | 3.0 | 1.1 | |
| St. 15 | 1 | 0.0 | 0.9 | 0.0 | 0.942 | 3.4 | 0.0 | 3.0 | 0.0 | 0.120 |
| | 2 | 10.0 | 7.4 | 8.3 | | 17.2 | 8.8 | 0.0 | 6.5 | |
| | 3 | 30.0 | 26.2 | 30.6 | | 34.5 | 25.9 | 30.3 | 29.3 | |
| | 4 | 44.3 | 51.5 | 50.0 | | 34.5 | 51.9 | 51.5 | 48.9 | |
| | 5 | 15.7 | 14.0 | 11.1 | | 10.3 | 13.4 | 15.2 | 15.2 | |

* statistically significant ($p < 0.05$, $\chi^2$ test). Data presented as percentage (%).

Despite the existence of different sub-social groups in Turkey, the Turkish family structure is mostly patriarchal. The members of the family perceive this structure as different and unique when compared to the rest of society. For this reason, women who are subjected to DVA often accept the situation in silence, fearful of further damaging their families. In cases where clinical findings point to DVA, the victim's reluctance to disclose the truth may result in health professionals avoiding formal reporting. On the other hand, in cases where DVA is disclosed, they could, unfortunately, be ignored and disregarded by some medical, legal and social authorities due to the belief that reporting the issue might negatively impact the image of the Turkish family structure. Therefore, it should be anticipated that the sociocultural characteristics of the society in which the participants were brought up will also be reflected in their responses.

A substantial number of women who have experienced abuse require dental treatment. Dentists, given their focus on the orofacial area, play an important role in identifying such women and ensuring that they receive the necessary assistance.[29,30] Many studies have shown that training on this subject improves dentists' understanding and changes their attitude towards DVA.[31,32] Dentists need to pay attention to the signs of DVA and provide support to those who have been victimized. It has been reported that healthcare professionals who have encountered cases of DVA in their professional practice have

Table 7. Correlation analysis

| Statement | Age | | Duration of professional practice | |
|---|---|---|---|---|
| | *r* | *p*-value | *r* | *p*-value |
| St. 1 | –0.115 | 0.026* | –0.082 | 0.114 |
| St. 2 | 0.069 | 0.186 | 0.032 | 0.543 |
| St. 3 | 0.059 | 0.259 | 0.048 | 0.357 |
| St. 4 | –0.036 | 0.490 | –0.001 | 0.982 |
| St. 5 | –0.06 | 0.248 | –0.038 | 0.462 |
| St. 6 | –0.078 | 0.132 | –0.041 | 0.436 |
| St. 7 | –0.181 | <0.001** | –0.145 | 0.005** |
| St. 8 | –0.201 | <0.001** | –0.156 | 0.003** |
| St. 9 | –0.184 | <0.001** | –0.128 | 0.014* |
| St. 10 | –0.079 | 0.131 | –0.077 | 0.137 |
| St. 11 | –0.084 | 0.105 | –0.059 | 0.256 |
| St. 12 | –0.034 | 0.510 | 0.004 | 0.939 |
| St. 13 | –0.201 | <0.001** | –0.138 | 0.008** |
| St. 14 | 0.013 | 0.799 | 0.033 | 0.526 |
| St. 15 | –0.121 | 0.020* | –0.070 | 0.179 |

* $p < 0.05$; ** $p < 0.01$; r – Spearman's rho.

difficulty reporting these cases to the relevant authorities.[10] Although approx. 6,000,000 people in the United States are affected by this problem, the actual reporting rate is quite low.[33] While there are many causes for this under-reporting rate, the main reason is the inadequate training of healthcare professionals in recognizing and diagnosing DVA. In a study by McDowell et al., it was found that physicians were uncertain about the procedure for reporting suspected cases of abuse when they encountered them.[34] As a necessary consequence, the authors recommend that educational courses and practical training with a multidisciplinary perspective be introduced into the curriculum to establish or improve dentists' proficiency in assessing the psychological, social, legal, and physical aspects of sensitive cases such as DVA.

## Limitations

Despite the strengths of our study, it has some limitations. Given that the target population was dentists and the objective was to achieve a high participation rate, not all aspects of DVA against women were assessed. Although we informed all participants that the study was designed not to evaluate individual knowledge levels, the structure of self-reported responses in the study could not entirely exclude the idealistic approach. Considering that the number of female dentists in Turkey is quite high, another limitation of this study is the possibility of selective perception bias due to the high proportion of female participants. The Turkish family structure and sociocultural factors may have influenced the responses and introduce bias. In this regard, the potential influence of geographical and sociocultural factors on the external validity of the results should be considered. Thus, the outcomes may not be generalizable to dentists in other countries.

# Conclusions

In comparison to other healthcare professionals, the level of responsibility attributed to dentists in the context of DVA against women is quite low. This situation calls for interdisciplinary training. When confronted with suspicious situations concerning violence, dentists do not fully comprehend their potential role in preventing more serious assaults. No study has shown that women who have been victims of DVA seek or request assistance from dentists. Dentists are the primary healthcare professionals responsible for recognizing and reporting head, neck and maxillofacial injuries to the authorities. The identification of women who have been victims of DVA enables dentists to provide the necessary care and protection to such individuals.

## Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee for Non-interventional Research of Istanbul Aydin University, Turkey (decision No. 2021/648).

## 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

Suleyman Emre Meseli https://orcid.org/0000-0002-8922-155X
Hulya Yildiz https://orcid.org/0000-0002-0847-8223


### References


1. World Health Organization (WHO). Global and regional estimates of violence against women: Prevalence and health effects of intimate partner violence and non-partner sexual violence. https://www.who.int/publications/i/item/9789241564625. Accessed July 23, 2023.
2. Till-Tentschert U. The relation between violence experienced in childhood and women's exposure to violence in later life: Evidence from Europe. *J Interpers Violence*. 2017;32(12):1874–1894. doi:10.1177/0886260517698952
3. Sugg N. Intimate partner violence: Prevalence, health consequences, and intervention. *Med Clin North Am*. 2015;99(3):629–649. doi:10.1016/j.mcna.2015.01.012
4. Bermea AM, Slakoff DC, Goldberg AE. Intimate partner violence in the LGBTQ+ community: Experiences, outcomes, and implications for primary care. *Prim Care*. 2021;48(2):329–337. doi:10.1016/j.pop.2021.02.006

5. Chuemchit M, Chernkwanma S, Somrongthong R, Spitzer DL. Thai women's experiences of and responses to domestic violence. *Int J Womens Health*. 2018;10:557–565. doi:10.2147/IJWH.S172870
6. Pallitto CC, García-Moreno C, Jansen HAFM, et al. Intimate partner violence, abortion, and unintended pregnancy: Results from the WHO Multi-country Study on Women's Health and Domestic Violence. *Int J Gynaecol Obstet*. 2013;120(1):3–9. doi:10.1016/j.ijgo.2012.07.003
7. Patra P, Prakash J, Patra B, Khanna P. Intimate partner violence: Wounds are deeper. *Indian J Psychiatry*. 2018;60(4):494–498. doi:10.4103/psychiatry.IndianJPsychiatry_74_17
8. Cavalcanti AFC, da Costa SRR, Goncharuk-Khomyn M, Cavalcanti AL. Maxillofacial injuries in Brazilian children and adolescents victims of physical violence: A single forensic center analysis. *Pesqui Bras Odontopediatria Clín Integr*. 2020;20:e568. doi:10.1590/pboci.2020.132
9. Garbin CAS, de Guimarães e Queiroz APD, Rovida TAS, Garbin AJI. Occurrence of traumatic dental injury in cases of domestic violence. *Braz Dent J*. 2012;23(1):72–76. doi:10.1590/s0103-64402012000100013
10. Caldas IM, Grams AC, Afonso A, Magalhães T. Oral injuries in victims involving intimate partner violence. *Forensic Sci Int*. 2012;221(1–3):102–105. doi:10.1016/j.forsciint.2012.04.010
11. Mascarenhas AK, Deshmukh A, Scott T. New England, USA dental professionals' attitudes and behaviours regarding domestic violence. *Br Dent J*. 2009;206(3):E5. doi:10.1038/sj.bdj.2009.7
12. Kara P, Akçayüzlü Ö, Gür AÖ, Nazik E. Determination of knowledge levels associated with recognition of violence against women of health professionals. *DU Sağlık Bil Enst Derg*. 2018;8(3):115–122. doi:10.33631/duzcesbed.441772
13. Lawshe CH. A quantitative approach to content validity. *Pers Psychol*. 1975;28(4):563–575. doi:10.1111/j.1744-6570.1975.tb01393.x
14. Chhikara P, Jakhar JK, Malik A, Singla K, Dhattarwal SK. Domestic violence: The dark truth of our society. *J Indian Acad Forensic Med*. 2013;35(1):71–75. https://www.researchgate.net/publication/287939259_Domestic_violence_The_dark_truth_of_our_society. Accessed July 23, 2023.
15. Raja S, Rajagopalan CF, Kruthoff M, Kuperschmidt A, Chang P, Hoersch M. Teaching dental students to interact with survivors of traumatic events: Development of a two-day module. *J Dent Educ*. 2015;79(1):47–55. doi:10.1002/j.0022-0337.2015.79.1.tb05856.x
16. Saleem MN, Raja HZ, Shakoor A, Rashid H, Nasir H, Yusuf E. Knowledge, attitude, and practices of dental practitioners regarding domestic violence in Pakistan. *Inquiry*. 2021;58:469580211060799. doi:10.1177/00469580211060799
17. de Macedo Bernardino Í, Santos LM, Ferreira AVP, de Almeida Lima TLM, da Nóbrega LM, d'Avila S. Intimate partner violence against women, circumstances of aggressions and oral-maxillofacial traumas: A medical-legal and forensic approach. *Leg Med (Tokyo)*. 2018;31:1–6. doi:10.1016/j.legalmed.2017.12.001
18. Caldas IM, Magalhães T, Afonso A, Matos E. The consequences of orofacial trauma resulting from violence: A study in Porto. *Dent Traumatol*. 2010;26(6):484–489. doi:10.1111/j.1600-9657.2010.00936.x
19. Saddki N, Suhaimi AA, Daud R. Maxillofacial injuries associated with intimate partner violence in women. *BMC Public Health*. 2010;10:268. doi:10.1186/1471-2458-10-268
20. Kishore Kumar RV, Devireddy SK, Gali RS, Chaithanyaa N, Sridhar. Clinician's role in the management of soft tissue injuries of the face: A clinical paper. *J Maxillofac Oral Surg*. 2013;12(1):21–29. doi:10.1007%2Fs12663-012-0352-z
21. UN Women. Global Database on Violence against Women. https://evaw-global-database.unwomen.org/-/media/files/un%20women/vaw/full%20text/asia/turkey%20-%20national%20research%20on%20domestic%20violence%20against%20women%202008/turkey%20-%20national%20research%20on%20domestic%20violence%20against%20women%202008.pdf?vs=5404. Accessed July 23, 2023.
22. Yüksel-Kaptanoğlu I, Çavlin A, Ergocmen Akadlı B, eds. Research on Domestic Violence Against Women in Turkey. Ankara, Turkey: Republic of Turkey Ministry of Family and Social Policies; 2015. https://openaccess.hacettepe.edu.tr/xmlui/handle/11655/23338. Accessed August 14, 2024.
23. McCauley J, Yurk RA, Jenckes MW, Ford DE. Inside "Pandora's box": Abused women's experiences with clinicians and health services. *J Gen Intern Med*. 1998;13(8):549–555. doi:10.1046/j.1525-1497.1998.00166.x
24. Chamberlain L, Perham-Hester KA. The impact of perceived barriers on primary care physicians' screening practices for female partner abuse. *Women Health*. 2002;35(2–3):55–69. doi:10.1300/J013v35n02_04
25. Richardson J, Coid J, Petruckevitch A, Chung WS, Moorey S, Feder G. Identifying domestic violence: Cross sectional study in primary care. *BMJ*. 2002;324(7332):274. doi:10.1136/bmj.324.7332.274
26. Schuler ME, Nair P. Witnessing violence among inner-city children of substance-abusing and non-substance-abusing women. *Arch Pediatr Adolesc Med*. 2001;155(3):342–346. doi:10.1001/archpedi.155.3.342
27. Rodriguez MA, Quiroga SS, Bauer HM. Breaking the silence. Battered women's perspectives on medical care. *Arch Fam Med*. 1996;5(3):153–158. doi:10.1001/archfami.5.3.153
28. Love C, Gerbert B, Caspers N, Bronstone A, Perry D, Bird W. Dentists' attitudes and behaviors regarding domestic violence. The need for an effective response. *J Am Dent Assoc*. 2001;132(1):85–93. doi:10.14219/jada.archive.2001.0032
29. da Nóbrega LM, de Macedo Bernardino Í, Barbosa KGN, Lira E Silva JA, de Lima Targino Massoni AC, d'Avila S. Pattern of oral-maxillofacial trauma from violence against women and its associated factors. *Dent Traumatol*. 2017;33(3):181–188. doi:10.1111/edt.12327
30. Tijaden P, Thoennes N. Prevalence, incidence, and consequences of violence against women: Findings from the national violence against women survey. National Institute of Justice, Centers for Disease Control and Prevention. 1998. Accessed July 23, 2023.
31. Danley D, Gansky SA, Chow D, Gerbert B. Preparing dental students to recognize and respond to domestic violence: The impact of a brief tutorial. *J Am Dent Assoc*. 2004;135(1):67–73. doi:10.14219/jada.archive.2004.0022
32. Hsieh NK, Herzig K, Gansky SA, Danley D, Gerbert B. Changing dentists' knowledge, attitudes and behavior regarding domestic violence through an interactive multimedia tutorial. *J Am Dent Assoc*. 2006;137(5):596–603. doi:10.14219/jada.archive.2006.0254
33. Rodriguez MA, Bauer HM, McLoughlin E, Grumbach K. Screening and intervention for intimate partner abuse: Practices and attitudes of primary care physicians. *JAMA*. 1999;282(5):468–474. doi:10.1001/jama.282.5.468
34. McDowell JD, Kassebaum DK, Fryer GE. Recognizing and reporting dental violence: A survey of dental practitioners. *Spec Care Dentist*. 1994;14(2):49–53. doi:10.1111/j.1754-4505.1994.tb01099.x

Original paper

# Effect of different intraoral scanners and post-space depths on the trueness of digital impressions

Marwa Emam[1,A–F], Lomaya Ghanem[2,B,F], Hoda M. Abdel Sadek[1,A–D,F]

[1] Department of Fixed Prosthodontics, Faculty of Dentistry, Ain Shams University, Cairo, Egypt
[2] Department of Fixed Prosthodontics and Conservative Dentistry, Faculty of Oral and Dental Medicine, Misr International University, Cairo, Egypt

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(4):577–584

**Address for correspondence**
Marwa Emam
E-mail: marwaemam@asfd.asu.edu.eg

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
The authors would like to thank Dr. Yasmine Ashraf for her assistance with the Geomagic® software.

Received on February 24, 2023
Reviewed on March 15, 2023
Accepted on March 23, 2023

Published online on April 18, 2023

## Abstract

**Background.** The trueness of intraoral scanners (IOSs) has been evaluated in many clinical situations. However, the tests of their performance when scanning post-space preparations are still lacking.

**Objectives.** The aim of the present study was to compare the trueness of the digital impressions of post spaces with different depths, captured by means of different IOSs.

**Material and methods.** Digital impressions of teeth ($N = 16$) with post spaces of depths of 8 mm and 10 mm were captured. Three IOSs were used, including Primescan AC, Medit i500 and CS 3600. The STL files were compared to the files obtained from the traditional impression scanning performed with an InEos X5 desktop scanner. Then, reverse engineering software measured the trueness values, which were analyzed using the two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. The significance level was set at $p < 0.05$.

**Results.** Significant differences were found between the scanners in terms of root mean square (RMS) values ($p < 0.001$). The highest RMS value was found for CS 3600 (0.30 ±0.11 mm), followed by Primescan AC (0.26 ±0.09 mm), while the lowest value was found for Medit i500 (0.18 ±0.05 mm). The 8-millimeter-deep post spaces had a significantly higher RMS value than the 10-millimeter-deep ones (0.28 ±0.10 mm and 0.21 ±0.09 mm, respectively) ($p = 0.009$).

**Conclusions.** The Medit i500 scanner showed the highest post-space digital impression trueness as compared to Primescan AC and CS 3600. In the digital impressions captured with CS 3600, the 10 mm post-space depth had higher trueness than the 8 mm depth. Moreover, CS 3600 was less able to capture the full length of both the 8 mm and 10 mm post-space depths than Primescan AC and Medit i500.

**Keywords:** CAD/CAM, root canal preparation, scanners, post-and-core technique

**Cite as**
Emam M, Ghanem L, Abdel Sadek HM. Effect of different intraoral scanners and post-space depths on the trueness of digital impressions. *Dent Med Probl.* 2024;61(4):577–584. doi:10.17219/dmp/162573

**DOI**
10.17219/dmp/162573

## Introduction

Most endodontically treated teeth require a core build-up with restorative materials to restore the lost tooth structure, and a post inserted inside the root canal to retain the core.[1] Ideally, the post should be bonded with a thin uniform layer of resin cement. A thick cement layer leads to polymerization contraction and creates internal stresses that cause cement fractures and the debonding of the post.[1] Moreover, root canals may show anomalies that affect the cement layer thickness, though custom-made posts have a shape that is more similar to the actual anatomy of the root canal. Conventionally, customized posts and cores are constructed in a two-step procedure that involves taking an impression, followed by fabrication in the dental laboratory.[2–5] Conventional impressions are taken with the use of elastomeric impression materials. Indeed, the accuracy and biocompatibility of these materials have been established.[6] Nonetheless, their use is related to several inconveniences, both from the operator's and the patient's standpoint, as it can cause anxiety, discomfort and nausea.[5,7]

The launch of computer-aided design and computer-aided manufacturing (CAD/CAM) technologies has revolutionized the processing of dental restorations.[8] A significant aspect of CAD/CAM are the scanners used, available as intraoral or extraoral devices. An intraoral scanner (IOS) provides direct imaging, while an extraoral scanner provides indirect imaging by scanning the master cast poured from the analog impression.[9] A digital impression created with IOS can be easily repeated and easily transferred to the dental laboratory, and the process itself is characterized by real-time model visualization and time efficiency.[7,10–14] However, digital systems have drawbacks, such as the significant cost of the initial purchase and the ongoing maintenance, difficulty in detecting deep margins, and the fact that blood and saliva hinder data capture.[15] Nonetheless, the dimensional accuracy of digital models generated by intraoral scanning is deemed high in comparison with the desktop scanning of conventional impressions.[16–22]

Conventional impressions can be digitalized for CAD/CAM post and core fabrication after being sprayed with an anti-reflective coating. Furthermore, the introduction of IOS has enabled the direct scanning of intracanal post-space preparations without the use of conventional impression techniques.[23,24] Regardless, limitations related to the intraoral environment (oral fluids) and IOS motion, especially in the posterior region, should be taken into consideration.[1]

Trueness is defined as 'the ability of a measurement to match the actual value'.[25,26] The trueness of IOS is affected by the scan pattern, the properties of the scanned object, the distance between the scanner and the object, and the size of the scanner head and lightbox.[27–32] The three-dimensional (3D) trueness of a virtual model can be evaluated by calculating its root mean square (RMS) value.[33] The comparative analysis of 3D data can be performed by using a coordinate-measuring machine[34] or metrology software,[26] which has been adopted from engineering and used to evaluate IOS and conventional impressions.[26] Meanwhile, precision is defined as 'the ability of a measurement to be consistently reproduced'.[26] Although trueness and precision are independent and each can be assessed separately, when both parameters are measured, they can be used to evaluate the accuracy of IOS.

The ability of different scanners to accurately read the post-space depth is not clear yet. Only a few studies have assessed the effect of the post-space depth on digital and conventional silicon impression accuracy.[1,35] Therefore, the present study aimed to compare the trueness of the digital impressions of post spaces with different depths, captured by means of different IOSs. The null hypothesis was that trueness would not differ according to the post-space depth or the type of IOS used.

## Material and methods

The study was approved by the ethics committee at the Faculty of Dentistry of the Ain Shams University, Cairo, Egypt (FDASU-REC ER032238).

A total of 16 ($N$ = 16) single straight-rooted human teeth – maxillary incisors and mandibular premolars – free of cracks and caries were selected. A priori power analysis was performed using the G*Power software, v.3.1.9.7 (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower), based on the results of a previous study.[36] The minimum group sample size was determined to be 2 (power = 0.95; effect size = 8.01), and an increase in the group sample size could increase the study power. The sample teeth were collected so that their root anatomy and dimensions would be similar. The teeth were cleaned and stored in distilled water throughout the sampling period before being decoronated by using a diamond disk mounted on a straight handpiece at 2 mm coronal to the cementoenamel junction and perpendicular to the long axis of the tooth. A routine root canal treatment procedure was carried out and periapical radiographs were used for inspection. The roots were randomly assigned into 2 groups ($n$ = 8) according to the depth of post-space drilling, at either 8 mm (group 8) or 10 mm (group 10). Each root was mounted vertically in an acrylic block by using self-cured acrylic resin (Acrostone Dental & Medical Supplies, Cairo, Egypt) (Fig. 1). A single operator prepared standardized post spaces for all teeth by using a tapered post drill #1.6 mm (Olipost Drill, Olident, Cracow, Poland).

Digital and traditional impressions were taken for each sample. Digital impressions were obtained first,

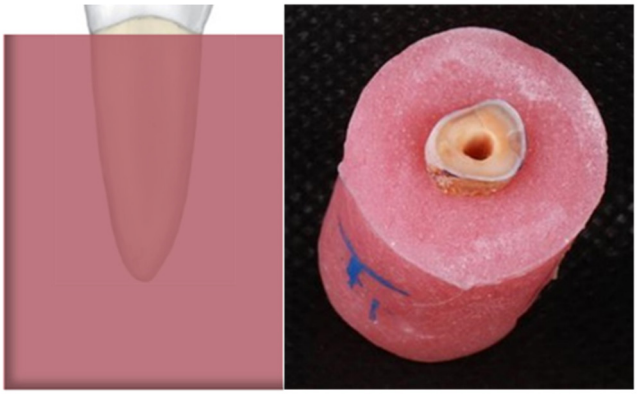

Fig. 1. Diagram and photo of the sample mounted in an acrylic block

as the silicone material remaining after applying the conventional impression technique might affect the post-space depth, and thus the accuracy of the digital impression data. The digital impressions of the post spaces were created with 3 different IOSs, including Primescan AC with Connect™ Software (Dentsply Sirona, Bensheim, Germany), Medit i500 (Medit Corp., Seoul, South Korea) and CS 3600 (Carestream Dental, Stuttgart, Germany). The scanner systems, manufacturers, software versions, and scanning technologies are listed in Table 1.

An occlusal notch was marked buccally as a starting point, the samples were fixed in place and all scanners were rotated clockwise. Digital scanning was performed at room temperature by an experienced operator to minimize operator experience bias.[37] STL files were generated from each IOS for all samples. Traditional impressions were taken with polyvinyl siloxane (SwissTEC HydroXtreme; Coltène/Whaledent, Altstätten, Switzerland), using a single-step two-material impression technique (Fig. 2).

To evaluate the trueness of the IOS reference, STL files were created by scanning each impression with an extraoral InEos X5 desktop scanner (Dentsply Sirona, Charlotte, USA), which is a highly accurate laboratory scanner that uses the digital stripe projection scanning technology with blue light, with each impression fixed separately to the five-axis robotic arm of the scanner.

Table 1. Scanner systems, manufacturers, software versions, and scanning technologies of the scanners used in the study

| System | Manufacturer | Software | Technology |
|---|---|---|---|
| Primescan AC | Dentsply Sirona, Bensheim, Germany | CEREC 4.5 | confocal microscopy |
| Medit i500 | Medit Corp., Seoul, South Korea | Medit Link 2.1.2 | dual camera optical triangulation |
| CS 3600 | Carestream Dental, Stuttgart, Germany | CS ScanFlow 1.0.5 | active triangulation |
| InEos X5 | Dentsply Sirona, Charlotte, USA | inLab 15 | optical blue structured light |

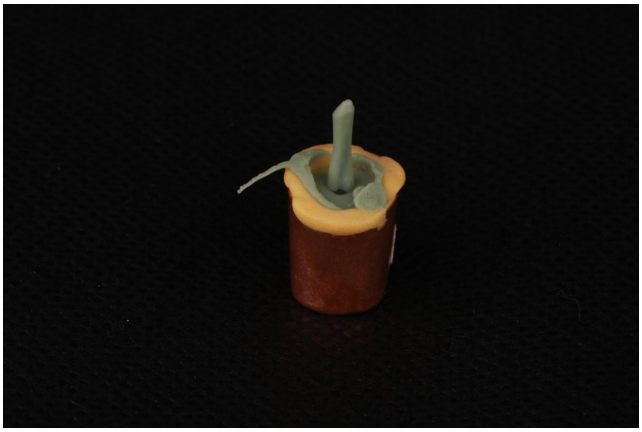

Fig. 2. Polyvinyl siloxane impression of a post space, ready for scanning with a desktop scanner

The trueness of the IOS was evaluated using reverse engineering software (Geomagic® Control X™ 2018; 3D Systems Manufacturing, Rock Hill, USA). The reference standard scan model was first trimmed to remove irrelevant data points and leave only the post-space data, which needed to be aligned. The unnecessary data points were excluded from the comparison with the test scans. Then, the "resegmenting" tool was used to manually segment the reference model, which enabled the restriction of deviation calculations to custom datasets. Each IOS scan file was imported, and then superimposed onto the reference model by using the initial alignment and the best-fit alignment for trueness measurements. The software best-fit alignment algorithm used the iterative closest-point procedure to align the 3D digital data of the test files and the reference files, which is the industry standard. After alignment, the "3D compare" function enabled the automatic isolation and comparison of substrate regions for the deviation computation of all locations of interest in post-space regions. The color-coded photographs of the model revealed the degree and pattern of the deviation of the 3D model. Darker blue signified a negative or inward deviation, while darker red signified a positive or outward deviation of the test model (Fig. 3).

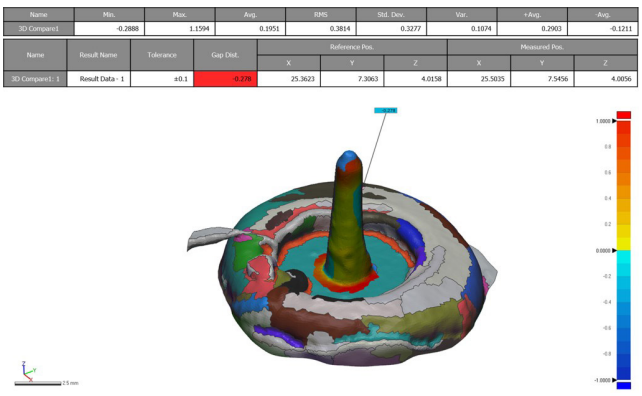

Fig. 3. Three-dimensional (3D) comparison of the superimposed test and reference post scans, showing the color map and the root mean square (RMS) value

Trueness was expressed as RMS, and the square of the phase difference between several points in 3D space was calculated (X-axis, Y-axis and Z-axis). The sum of these squares was then divided by the number of points, and the RMS was calculated as the square root of this value, using the following formula (Equation 1):

$$RMS = \frac{1}{\sqrt{n}} \times \sqrt{\sum_{i=1}^{n}(x_{1i} - x_{2i})^2} \quad (1)$$

where:
$x_{1i}$ – measurement of point i on the reference scan;
$x_{2i}$ – measurement of point i on the test scan; and
$n$ – total number of points measured in each analysis.

The RMS value may be employed to assess how different from zero the deviation between 2 different sets of data is. The lower the RMS value, the better the 3D agreement of the superimposed data.[33]

As for the length measurement with regard to the post-space depth, the STL files of the tested specimens were imported to the software individually before the "2D length measurement" tool was selected. To get the length of the post-space depth captured by each scanner, the distance from the selected point on the occlusal surface (the occlusal notch) to the apical end of the post scan was measured (Fig. 4).

## Statistical analysis

Statistical analysis was conducted with the use of the R statistical analysis software, v. 4.1.2 for Windows (R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). Numerical data was presented as mean and standard deviation ($M \pm SD$). The normality of data was assessed using the Shapiro–Wilk test, and Levene's test determined the homogeneity of variance. The data showed a parametric distribution and variance homogeneity. The trueness values were analyzed for the effects of the post-space depth and the scanner type by means of the two-way analysis of variance (ANOVA), followed by Tukey's post-hoc test. The comparison of the post-scan length with the post-space depth was performed utilizing the one-sample $t$ test. The correlation between trueness and the post-scan length was analyzed using Spearman's rank-order correlation coefficient. Intergroup comparisons utilized the one-way ANOVA, followed by Tukey's post-hoc test. The significance level was set at $p < 0.05$ for all tests.

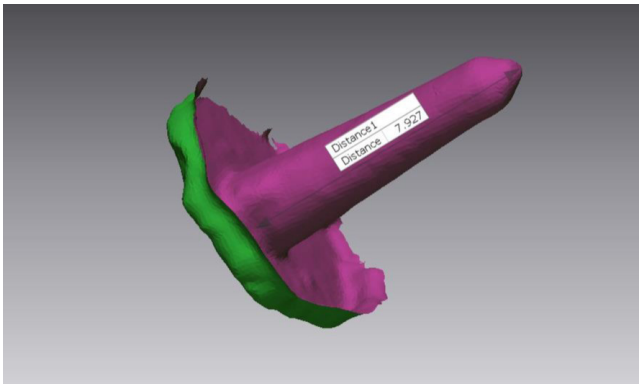


Fig. 4. Measurement of the post-scan length

# Results

Table 2 presents the significant effects of both the post-space depth and the scanner type on the RMS values ($p = 0.009$ and $p < 0.001$, respectively), though the interaction between the independent variables had no significant effect ($p = 0.178$).

Significant differences were found between the scanners in terms of RMS values ($p < 0.001$). The highest RMS value for trueness was found with CS 3600 (0.30 ±0.11 mm), followed by Primescan AC (0.26 ±0.09 mm), while the lowest value was found with Medit i500 (0.18 ±0.05 mm). In addition, the samples with 8-millimeter-deep post spaces had a significantly higher RMS value than those with 10-millimeter-deep post spaces (0.28 ±0.10 mm and 0.21 ±0.09 mm, respectively) ($p = 0.009$).

The post-hoc pairwise comparisons showed that the RMS trueness value was significantly lower for Medit i500 as compared to other scanners ($p < 0.001$).

The intergroup comparisons of the RMS values for trueness, presented in Table 3 and Fig. 5, showed significant differences in the RMS values between different groups ($p < 0.001$). The highest value was found for the CS 3600 group 8 (0.33 ±0.09 mm), followed by the Primescan AC group 8 (0.31 ±0.07 mm), the CS 3600 group 10 (0.26 ±0.11 mm), and the Primescan AC group 10 (0.20 ±0.07 mm). The lowest values were found for the Medit i500 group 8 (0.18 ±0.03 mm) and group 10 (0.18 ±0.06 mm).

Table 2. Two-way ANOVA results for the root mean square (RMS) values for trueness

| Parameter | Sum of squares | df | Mean square | *f*-value | *p*-value |
|---|---|---|---|---|---|
| Post-space depth | 0.05 | 1 | 0.05 | 7.48 | 0.009* |
| Scanner type | 0.11 | 2 | 0.06 | 9.42 | <0.001* |
| Post-space depth and scanner type | 0.02 | 2 | 0.01 | 1.80 | 0.178 |
| Error | 0.26 | 42 | 0.01 | – | – |

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

**Table 3.** Intergroup comparisons in terms of root mean square (RMS) values for trueness

| Parameter | Group | | | | | | *p*-value |
|---|---|---|---|---|---|---|---|
| | CS 3600 group 8 | Medit i500 group 8 | Primescan AC group 8 | CS 3600 group 10 | Medit i500 group 10 | Primescan AC group 10 | |
| RMS [mm] | 0.33 ±0.09[a] | 0.18 ±0.03[c] | 0.31 ±0.07[a,b] | 0.26 ±0.11[b,c] | 0.18 ±0.06[c] | 0.20 ±0.07[b,c] | <0.001* |

Data presented as mean ± standard deviation (*M* ±*SD*). * statistically significant ($p < 0.05$); different superscript letters mean statistically significant differences.

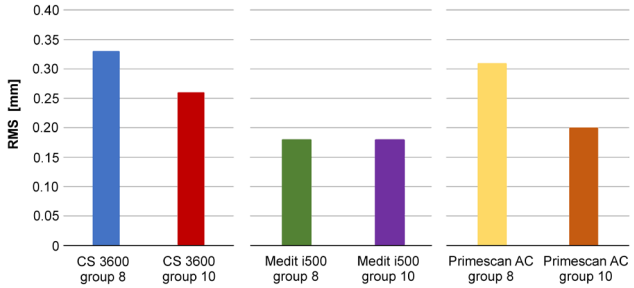


**Fig. 5.** Bar chart showing the intergroup comparisons in terms of root mean square (RMS) values for trueness

The post-hoc pairwise comparisons showed that the CS 3600 group 8 had a significantly higher RMS value than all other groups ($p < 0.001$), except for the Primescan AC group 8. In addition, they showed that the Primescan AC group 8 had a significantly higher RMS value than the Medit i500 groups 8 and 10 ($p < 0.001$).

The *M* ±*SD* values for the post-scan length in different groups are shown in Fig. 6. The one-sample *t* test results presented in Tables 4 and 5 show that only for CS 3600, for both the 8 mm and 10 mm post-space depths, there was a significant difference between the post-space depth and the post-scan length ($p < 0.05$).

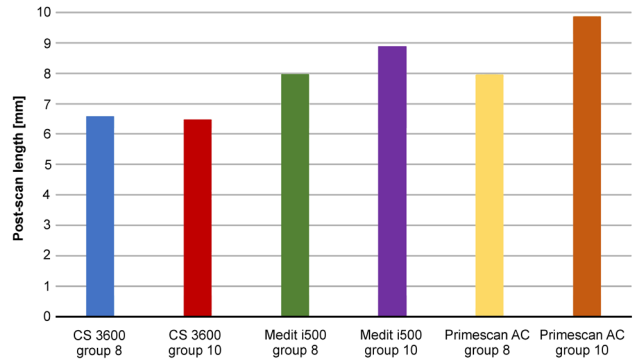


**Fig. 6.** Bar chart showing the mean values of the post-scan length in different groups

**Table 4.** Comparisons of the post-scan length with the post-space depth (8 mm) in different groups

| Post-space depth | Scanner | *MD* (95% *CI*) | df | *t*-value | *p*-value |
|---|---|---|---|---|---|
| 8 mm | CS 3600 | −1.42 (−2.35, −0.48) | 7 | 3.60 | 0.009* |
| | Medit i500 | −0.03 (−0.05, 0.02) | 7 | 2.17 | 0.066 |
| | Primescan AC | −0.04 (−0.13, 0.06) | 7 | 0.88 | 0.404 |

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

**Table 5.** Comparisons of the post-scan length with the post-space depth (10 mm) in different groups

| Post-space depth | Scanner | *MD* (95% *CI*) | df | *t*-value | *p*-value |
|---|---|---|---|---|---|
| 10 mm | CS 3600 | −3.53 (−4.30, −2.75) | 7 | 10.81 | <0.001* |
| | Medit i500 | −1.11 (−2.36, 0.14) | 7 | 2.10 | 0.074 |
| | Primescan AC | −0.12 (−0.34, 0.11) | 7 | 1.19 | 0.272 |

* statistically significant ($p < 0.05$).

## Discussion

Accessibility is generally hindered when scanning an intracoronal restoration design, such as an inlay, as compared to extracoronal designs; it poses a great problem especially in the case of intraradicular preparations.[38] The present study involved the scanning of 2 post-space depths of 8 mm and 10 mm with the use of 3 IOSs, and evaluated the trueness of the devices against a reference extraoral five-axis InEos X5 desktop scanner. The accuracy of its results was verified to be within 2.1 µm, according to ISO 12836:2015.[39–43]

This in vitro study investigated 3 IOSs using different imaging techniques. Primescan AC represents video-rate confocal microscopy, Medit i500 uses video-type scanning based on the triangulation technology and CS 3600 uses video-type scanning active triangulation. All the techniques acquire images with the aid of light and do not require surface coating with powder.[44]

The obtained results necessitated the rejection of the null hypothesis, as they showed significant differences in the trueness of IOSs. Regarding the RMS values, they were higher at 8 mm than at 10 mm, and higher trueness was acquired at the 10 mm depth only in the case of the CS 3600 scanner. A tapered post drill was used for creating post spaces, so the longer the post space, the wider the entrance. This may have led to an increased amount of IOS light entering the post space.[1] Moreover, the CS 3600 scanner had a low scanning depth, which the manufacturer assumed to be up to 12 mm, as compared to the 20 mm for Primescan AC and a range of 12–21 mm (a default depth of 18.5 mm) for Medit i500.[45–47] The scanning depth was assumed to affect both the feasibility of scanning and the accuracy of the scan data. Besides, the use of low-scanning-depth IOSs is related to a long learning curve, since the operator has to keep a distance from the scanned teeth while watching a computer display. When the maximum depth the IOS can reach

is shallow, image acquisition may not be possible in narrow post-space preparations.[32] These findings disagree with a study of Gurpinar and Tak, who investigated and compared the accuracy of different IOSs for scanning different pulpal chamber extension depths, and concluded that deep pulpal chamber extensions of endocrown restorations could negatively affect scanning accuracy.[48] Moreover, Pinto et al. concluded that the scanning effectiveness of the 3Shape IOS was insufficient for post-space impressions, especially for narrow root canals.[1]

Noticeable and significant differences were found for the RMS values between the scanners, regarding the trueness of the captured data. The CS 3600 scanner displayed the highest RMS value and the lowest trueness, while Medit i500 showed higher trueness, followed by Primescan AC. This could be attributed to the different scanning technologies, designs, techniques, and light intensity of each IOS system. The CS 3600 scanner uses a video sequence system, while Medit i500 stitches images. Meanwhile, Primescan AC has been described to use high-frequency contrast analysis as a patent scanning principle. However, various scanning strategies are not clearly explained by the manufacturers.[49]

As a clinically appropriate cement layer thickness has been established to be between 250 µm and 500 µm,[50] and all the IOSs investigated in this study showed RMS values ≤330 µm, the cement layer was considered clinically acceptable in all cases.

Regarding the post-space depth scans, the results showed significant differences for 8 mm and 10 mm, with the greatest mean difference between the post-scan length and the post-space depth in the case of CS 3600, for both group 8 and group 10. One of the main factors affecting full-depth recording and the trueness of the IOS is the capture box, which is the area in the scanner tip that captures the scanned object in each image. All IOSs require the projection of a sufficient amount of light to the point of interest before it is reflected and recorded. Therefore, a large capture box is preferred for the light to reach deeply for long post-space preparations, as a small capture box requires more stitching or connecting image files, which results in more errors.[51] The field of view was the smallest in CS 3600 (13 mm × 13 mm), as compared to Primescan AC (16 mm × 16 mm ) and Medit i500 (14 mm × 13 mm).[45–47] The results are in agreement with Elter et al., who concluded that Primescan AC could capture a digital post-space impression when the drilled post-space depth was less than 14 mm.[52]

Other factors influencing trueness, such as the operator's scanning skill, software and illumination, were not considered in this study. The fabrication and the assessment of the fit of the final restorations were also not performed, which might be considered a study limitation.

As the trueness of digital post-space impressions seems to be influenced by the geometry of the post space and the scanner type, Medit i500 and Primescan AC are preferable when recording the full length of the post-space depth to an acceptable degree in clinical practice; in the case of CS 3600, the discrepancy between the post-scan length and the post-space depth was too large, and the trueness RMS value was too high for the scanner to be clinically accepted.

# Conclusions

The Medit i500 scanner showed the highest post-space digital impression trueness as compared to Primescan AC and CS 3600. In the digital impressions captured with the CS 3600, the 10 mm post-space depth had higher trueness than the 8 mm depth. Furthermore, CS 3600 showed less ability to capture the full length of both the 8 mm and 10 mm post-space depths than Primescan AC and Medit i500.

## Ethics approval and consent to participate

The study was approved by the ethics committee at the Faculty of Dentistry of the Ain Shams University, Cairo, Egypt (FDASU-REC ER032238). All the procedures applied in the current study were performed in accordance with the relevant guidelines and regulations.

## Data availability

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

## Consent for publication

Not applicable.


### ORCID iDs

Marwa Emam https://orcid.org/0000-0003-0856-0689
Lomaya Ghanem https://orcid.org/0000-0003-4258-9346
Hoda M. Abdel Sadek https://orcid.org/0000-0001-7339-0861


### References


1. Pinto A, Arcuri L, Carosi P, et al. In vitro evaluation of the post-space depth reading with an intraoral scanner (IOS) compared to a traditional silicon impression. *Oral Implantol (Rome)*. 2017;10(4):360–368. doi:10.11138/orl/2017.10.4.360
2. Gomes GM, Gomes OM, Gomes JC, Loguercio AD, Calixto AL, Reis A. Evaluation of different restorative techniques for filling flared root canals: Fracture resistance and bond strength after mechanical fatigue. *J Adhes Dent*. 2014;16(3):267–276. doi:10.3290/j.jad.a31940
3. Baba NZ, Goodacre CJ, Daher T. Restoration of endodontically treated teeth: The seven keys to success. *Gen Dent*. 2009;57(6):596–603. PMID:19906612.
4. Morgano SM, Rodrigues AH, Sabrosa CE. Restoration of endodontically treated teeth. *Dent Clin*. 2004;48(2):397–416. doi:10.1016/j.cden.2003.12.011
5. Christopher VS, Ranjan M. Prevalence of age and sex in uses of custom made cast post in endodontically treated teeth – a retrospective study. *Int J Dentistry Oral Sci*. 2021;8(7):3257–3262. doi:10.19070/2377-8075-21000663

6. Berrendero S, Salido MP, Ferreiroa A, Valverde A, Pradíes G. Comparative study of all-ceramic crowns obtained from conventional and digital impressions: Clinical findings. *Clin Oral Investig*. 2019;23(4):1745–1751. doi:10.1007/s00784-018-2606-8
7. Christensen GJ. Impressions are changing: Deciding on conventional, digital or digital plus in-office milling. *J Am Dent Assoc*. 2009;140(10):1301–1304. doi:10.14219/jada.archive.2009.0054
8. Joda T, Zarone F, Ferrari M. The complete digital workflow in fixed prosthodontics: A systematic review. *BMC Oral Health*. 2017;17(1):124. doi:10.1186/s12903-017-0415-0
9. Güth JF, Keul C, Stimmelmayr M, Beuer F, Edelhoff D. Accuracy of digital models obtained by direct and indirect data capturing. *Clin Oral Investig*. 2013;17(4):1201–1208. doi:10.1007/s00784-012-0795-0
10. Pang J, Feng C, Zhu X, et al. Fracture behaviors of maxillary central incisors with flared root canals restored with CAD/CAM integrated glass fiber post-and-core. *Dent Mater J*. 2019;38(1):114–119. doi:10.4012/dmj.2017-394
11. Lee JH. Fabricating a custom zirconia post-and-core without a post-and-core pattern or a scan post. *J Prosthet Dent*. 2018;120(2):186–189. doi:10.1016/j.prosdent.2017.10.004
12. Libonati A, Di Taranto V, Gallusi G, Montemurro E, Campanella V. CAD/CAM customized glass fiber post and core with digital intraoral impression: A case report. *Clin Cosmet Investig Dent*. 2020;12:17–24. doi:10.2147/CCIDE.S237442
13. Yilmaz H, Aydin MN. Digital versus conventional impression method in children: Comfort, preference and time. *Int J Paediatr Dent*. 2019;29(6):728–735. doi:10.1111/ipd.12566
14. Tomita Y, Uechi J, Konno M, Sasamoto S, Iijima M, Mizoguchi I. Accuracy of digital models generated by conventional impression/plaster-model methods and intraoral scanning. *Dent Mater J*. 2018;37(4):628–633. doi:10.4012/dmj.2017-208
15. Jivănescu A, Bara A, Faur AB, Rotar RN. Is there a significant difference in accuracy of four intraoral scanners for short-span fixed dental prosthesis? A comparative in vitro study. *Appl Sci*. 2021;11(18):8280. doi:10.3390/app11188280
16. Wiranto MG, Engelbrecht WP, Tutein Nolthenius HE, van der Meer WJ, Ren Y. Validity, reliability, and reproducibility of linear measurements on digital models obtained from intraoral and cone-beam computed tomography scans of alginate impressions. *Am J Orthod Dentofacial Orthop*. 2013;143(1):140–147. doi:10.1016/j.ajodo.2012.06.018
17. Flügge TV, Schlager S, Nelson K, Nahles S, Metzger MC. Precision of intraoral digital dental impressions with iTero and extraoral digitization with the iTero and a model scanner. *Am J Orthod Dentofacial Orthop*. 2013;144(3):471–478. doi:10.1016/j.ajodo.2013.04.017
18. Hayashi K, Sachdeva AU, Saitoh S, Lee SP, Kubota T, Mizoguchi I. Assessment of the accuracy and reliability of new 3-dimensional scanning devices. *Am J Orthod Dentofacial Orthop*. 2013;144(4):619–625. doi:10.1016/j.ajodo.2013.04.021
19. Aragón ML, Pontes LF, Bichara LM, Flores-Mir C, Normando D. Validity and reliability of intraoral scanners compared to conventional gypsum models measurements: A systematic review. *Eur J Orthod*. 2016;38(4):429–434. doi:10.1093/ejo/cjw033
20. Anh JW, Park JM, Chun YS, Kim M, Kim M. A comparison of the precision of three-dimensional images acquired by 2 digital intraoral scanners: Effects of tooth irregularity and scanning direction. *Korean J Orthod*. 2016;46(1):3–12. doi:10.4041/kjod.2016.46.1.3
21. Grünheid T, McCarthy SD, Larson BE. Clinical use of a direct chairside oral scanner: An assessment of accuracy, time, and patient acceptance. *Am J Orthod Dentofacial Orthop*. 2014;146(1):673–682. doi:10.1016/j.ajodo.2014.07.023
22. Naidu D, Freer TJ. Validity, reliability, and reproducibility of the iOC intraoral scanner: A comparison of tooth widths and Bolton ratios. *Am J Orthod Dentofacial Orthop*. 2013:144(2):304–310. doi:10.1016/j.ajodo.2013.04.011
23. Moustapha G, AlShwaimi E, Silwadi M, Ounsi H, Ferrari M, Salameh Z. Marginal and internal fit of CAD/CAM fiber post and cores. *Int J Comput Dent*. 2019;22(1):45–53. PMID:30848254.
24. Jafarian Z, Moharrami M, Sahebi M, Alikhasi M. Adaptation and retention of conventional and digitally fabricated posts and cores in round and oval-shaped canals. *Int J Prosthodont*. 2020;33(1):91–98. doi:10.11607/ijp.6313
25. Vág J, Renne W, Revell G, et al. The effect of software updates on the trueness and precision of intraoral scanners. *Quintessence Int*. 2021;52(7):636–644. doi:10.3290/j.qi.b1098315
26. Nedelcu R, Olsson P, Nyström I, Thor A. Finish line distinctness and accuracy in 7 intraoral scanners versus conventional impression: An in vitro descriptive comparison. *BMC Oral Health*. 2018;18(1):27. doi:10.1186/s12903-018-0489-3
27. Hayama H, Fueki K, Wadachi J, Wakabayashi N. Trueness and precision of digital impressions obtained using an intraoral scanner with different head size in the partially edentulous mandible. *J Prosthodont Res*. 2018;62(3):347–352. doi:10.1016/j.jpor.2018.01.003
28. Kim MK, Kim JM, Lee YM, Lim YJ, Lee SP. The effect of scanning distance on the accuracy of intra-oral scanners used in dentistry. *Clin Anat*. 2019;32(3):430–438. doi:10.1002/ca.23334
29. Larson TD, Nielsen MA, Brackett WW. The accuracy of dual-arch impressions: A pilot study. *J Prosthet Dent*. 2002;87(6):625–627. doi:10.1067/mpr.2002.125180
30. Lim JH, Park JM, Kim M, Heo SJ, Myung JY. Comparison of digital intraoral scanner reproducibility and image trueness considering repetitive experience. *J Prosthet Dent*. 2018;119(2):225–232. doi:10.1016/j.prosdent.2017.05.002
31. Mennito AS, Evans ZP, Lauer AW, Patel RB, Ludlow ME, Renne WG. Evaluation of the effect scan pattern has on the trueness and precision of six intraoral digital impression systems. *J Esthet Restor Dent*. 2018;30(2):113–118. doi:10.1111/jerd.12371
32. Park JM. Comparative analysis on reproducibility among 5 intraoral scanners: Sectional analysis according to restoration type and preparation outline form. *J Adv Prosthodont*. 2016;8(5):354–362. doi:10.4047/jap.2016.8.5.354
33. Park GH, Son K, Lee KB. Feasibility of using an intraoral scanner for a complete-arch digital scan. *J Prosthet Dent*. 2019;121(5):803–810. doi:10.1016/j.prosdent.2018.07.014
34. Neshandar Asli H, Babaee Hemmati Y, Falahchai M. Three-dimensional accuracy of innovative implant-level impression techniques with plastic snap-on impression copings. *Dent Med Probl*. 2021;58(3):351–357. doi:10.17219/dmp/130089
35. Leven R, Schmidt A, Binder R, et al. Accuracy of digital impression taking with intraoral scanners and fabrication of CAD/CAM posts and cores in a fully digital workflow. *Materials (Basel)*. 2022;15(12):4199. doi:10.3390/ma15124199
36. Ashraf Y, Sabet A, Hamdy A, Ebeid K. Influence of preparation type and tooth geometry on the accuracy of different intraoral scanners. *J Prosthodont*. 2020;29(9):800–804. doi:10.1111/jopr.13202
37. Michelinakis G, Apostolakis D, Tsagarakis A, Kourakis G, Pavlakis E. A comparison of accuracy of 3 intraoral scanners: A single-blinded in vitro study. *J Prosthet Dent*. 2019;124(5):581–588. doi:10.1016/j.prosdent.2019.10.023
38. Waldecker M, Rues S, Rammelsberg P, Bömicke W. Accuracy of complete-arch intraoral scans based on confocal microscopy versus optical triangulation: A comparative in vitro study. *J Prosthet Dent*. 2020;126(3):414–420. doi:10.1016/j.prosdent.2020.04.019
39. Kim JE, Hong YS, Kang YJ, Kim JH, Shim JS. Accuracy of scanned stock abutments using different intraoral scanners: An in vitro study. *J Prosthodont*. 2019;28(7):797–803. doi:10.1111/jopr.13095
40. Park JM, Kim RJY, Lee KW. Comparative reproducibility analysis of 6 intraoral scanners used on complex intracoronal preparations. *J Prosthet Dent*. 2020;123(1):113–120. doi:10.1016/j.prosdent.2018.10.025
41. ISO 12836:2015. 2022. https://www.iso.org/standard/68414.html. Accessed October 16, 2022.
42. Dentsply Sirona. https://www.dentsplysirona.com/en-us/categories/lab/cad-cam-equipment-dental-lab/scan.html#technical-data. Accessed December 22, 2022.
43. Nulty AB. A comparison of full arch trueness and precision of nine intra-oral digital scanners and four lab digital scanners. *Dent J (Basel)*. 2021;9(7):75. doi:10.3390/dj9070075
44. Kim RJY, Benic GI, Park JM. Trueness of ten intraoral scanners in determining the positions of simulated implant scan bodies. *Sci Rep*. 2021;11(1):2606. doi:10.1038/s41598-021-82218-z
45. Dentsply Sirona. https://news.dentsplysirona.com/en/solutions-topics/primescan.html. Accessed November 6, 2022.
46. Medit Help Center. https://support.medit.com/hc/en-us/articles/360039964711-Adjusting-scan-depth. Accessed November 6, 2022.

47. Carestream 3600 Family Brochure. https://issuu.com/ozonecreative/docs/sua4078_cs3600usfamilybrochure. Accessed December 16, 2022.
48. Gurpinar B, Tak O. Effect of pulp chamber depth on the accuracy of endocrown scans made with different intraoral scanners versus an industrial scanner: An in vitro study. *J Prosthet Dent*. 2020;127(3):430–437. doi:10.1016/j.prosdent.2020.08.034
49. Shin SH, Yu HS, Cha JY, Kwon JS, Hwang CJ. Scanning accuracy of bracket features and slot base angle in different bracket materials by four intraoral scanners: An in vitro study. *Materials (Basel)*. 2021;14(2):365. doi:10.3390/ma14020365
50. Assif D, Bleicher S. Retention of serrated endodontic posts with a composite luting agent: Effect of cement thickness. *J Prosthet Dent*. 1986;56(6):689–691. doi:10.1016/0022-3913(86)90145-9
51. Amornvit P, Rokaya D, Peampring C, Sanohkan S. Confocal 3D optical intraoral scanners and comparison of image capturing accuracy. *Comput Mater Contin*. 2021;66(1):303–314. doi:10.32604/cmc.2020.011943
52. Elter B, Diker B, Tak Ö. The trueness of an intraoral scanner in scanning different post space depths. *J Dent*. 2022;127:104352. doi:10.1016/j.jdent.2022.104352

Original paper

# Nanoscale *Stolephorus* sp. powder fabrication using high-energy milling for bioactive materials in dentistry

Anastasia Elsa Prahasti[1,A,C,D,F], Tamara Yuanita[2,A,B,E,F], Retno Pudji Rahayu[3,C,E,F]

[1] Faculty of Dental Medicine, Airlangga University, Surabaya, Indonesia
[2] Department of Conservative Dentistry, Airlangga University, Surabaya, Indonesia
[3] Department of Oral and Maxillofacial Pathology, Airlangga University, Surabaya, Indonesia

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(4):585–592

**Address for correspondence**
Tamara Yuanita
E-mail: tamara.yuanita@klinikjurnal.com

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
The authors would like to thank Research Center for Physics, Indonesian Institute of Sciences, E-Layanan Sains, Jakarta, Indonesia, for scientific and technical support.

Received on January 30, 2023
Reviewed on February 19, 2023
Accepted on April 26, 2023

Published online on August 28, 2024

## Abstract

**Background.** The application of natural products in dentistry has been widely explored. Anchovy (*Stolephorus* in Latin) has been examined for its bioactive content (calcium, phosphorus and fluoride) as an agent for bone stimulation and tooth development, topical fluoridation and pulp capping. Ball milling has been used to prepare calcium oxide nanoparticles from snakehead fish bone.

**Objectives.** The aim of the study was to reduce the particle size of *Stolephorus* sp. powder to the nanoscale using high-energy ball milling for 8, 12 and 24 h, and to analyze the optimal milling time by comparing the powder characteristics.

**Material and methods.** The *Stolephorus* sp. were oven-dried at 50°C for 6 h, after which the entire fish were crushed into powder. The fish powder was produced by blending the material for 5 min and passing it through a 200-mesh sieve. The remaining dried fish was blended again for 5 min until it passed through the sieve. The top-down approach to the particle size reduction was performed using high-energy milling at 3 distinct time points (8, 12 and 24 h). The characteristics of the powder were evaluated using a particle size analyzer, a Fourier-transform infrared spectrometer (FTIR) and scanning electron microscopy–energy dispersive spectroscopy (SEM-EDS).

**Results.** The *Stolephorus* sp. powder contained 64.50% protein, 7,420 mg/kg sodium, 28,912 mg/kg calcium, and 1,924 mg/kg magnesium. The high-energy milling process resulted in a reduction of the particle size from the microscale to the nanoscale. The analysis of the average particle size and polydispersity index indicated that 24 h of milling showed the most optimal results. Furthermore, the functional groups exhibited no significant alteration at 3 milling times ($p \geq 0.05$, FTIR analysis).

**Conclusions.** The high-energy milling method has the potential to reduce the particle size of *Stolephorus* sp. powder to the nanoscale at the 8- and 24-h milling periods. The powder resulting from the 24-h milling process had a size of 789.3 ±170.7 nm, smooth size distribution, good size uniformity, a polydispersity index of 0.763, no significant change in organic and inorganic compound content, and a calcium/phosphorus ratio that was the closest to that of hydroxyapatite (HAp).

**Keywords:** nanoparticle, high-energy ball milling, good health and well-being, particle size reduction, *Stolephorus* sp.

**Cite as**
Prahasti AE, Yuanita T, Rahayu RP. Nanoscale *Stolephorus* sp. powder fabrication using high-energy milling for bioactive materials in dentistry. *Dent Med Probl.* 2024;61(4):585–592. doi:10.17219/dmp/163634

**DOI**
10.17219/dmp/163634

# Introduction

The development of new drugs is a long process, especially for those derived from natural products. However, natural products and traditional medicine have advantages in terms of diversity of chemical structures, biological activities and clinical experience.[1] The use of natural products has been widely explored in the field of dentistry. Anchovy (*Stolephorus* in Latin) has been studied for its bioactive content (calcium, phosphorus and fluoride) as an agent for bone stimulation and tooth development, topical fluoridation, and pulp capping.[2] *Stolephorus* sp. is classified within the order Clupeiformes, the Engraulidae family. It is a small pelagic fish, categorized as a renewable resource, with a maximum length of 40–145 mm. It has thin, quickly shedding scales and a silvery lateral line between its chest fin and tummy fin.[3] *Stolephorus* sp. can be used by extraction or as a pure powder (simplicia).[2] The *Stolephorus* sp. extract has superior activity compared to the pure powder in many compounds, whereas the pure powder has advantages in the number and complexity of bioactive compounds that work together.

*Stolephorus* sp. has been examined for its ability to increase hemoglobin levels in adolescent anemia.[4] The calcium and amino acid content of *Stolephorus* sp. is believed to play a role in the repair of cells and could benefit the field of dentistry. Calcium is also present in other foods, such as milk, bone soup, green vegetables, beans, tofu, and tempeh. Unfortunately, the content of fiber, phytic acid and oxalic acid in other food products inhibits the absorption of calcium. In addition, the lactose content of milk limits its consumption, particularly among individuals with lactose intolerance.[5]

It has been reported that *Stolephorus* sp. increases the proliferation of odontoblasts.[6] Another study mentioned its protein/amino acid benefits in bone function modulation.[7] Studies on the application of *Stolephorus* sp. cream to dental pulp perforation have demonstrated that the bioactive compound of *Stolephorus* sp. escalated the formation of reparative dentin compared to calcium hydroxide application.[8] Although, calcium hydroxide has been the gold standard for vital dental pulp perforation for decades. These findings indicate the potential for a new drug candidate for reparative dentin induction.

This study was considered necessary due to the increased success of vital pulp therapy using natural materials of nanosize to increase their effectiveness. The use of calcium hydroxide as the gold standard for vital pulp therapy is associated with the disadvantage of reparative dentin, which results in tunnel defects that lead to microleakage and can cause permanent pulp inflammation.[4,5] Previous studies have shown the potential of *Stolephorus* sp. powder to stimulate the growth of odontoblasts and produce reparative dentin, exhibiting superior efficacy compared to calcium hydroxide.[6,8] Our study aims to evaluate the technique for obtaining the nanosized *Stolephorus* sp. powder using high-energy milling. The results obtained at 8-, 12- and 24-h were compared according to the test characteristics.

Ball milling has been used to prepare calcium oxide nanoparticles from snakehead fish bone.[9] Previous studies have employed a chemical refining process, followed by calcination using a furnace and a ball milling process. In this study, ball milling was conducted on the whole fish powder that had been dried and blended without the application of any chemical processes. The duration of the milling process was modified to obtain nanoparticles.

Given the impact of nanoscale particles on the efficacy of *Stolephorus* sp., a reduction in the particle size could enhance the activity of *Stolephorus* sp. pure powder on cell targets.[10] Nanoparticles are obtained through a top-down approach using high-energy milling. The aim of the study is to reduce the particle size of *Stolephorus* sp. powder to a nanoscale using high-energy ball milling for 8, 12 and 24 h, and to analyze the optimal milling time by comparing the powder characteristics.

# Material and methods

## Research design

The anchovies used in the experiment were obtained from the fish market in Muara Karang, Jakarta, Indonesia, and identified as *Stolephorus* sp. by the Indonesian Institute of Sciences, Jakarta, Indonesia (ID No. 29825). The fish were oven-dried at 50°C for 6 h,[6] ground into a fine powder using a blender and sieved.[8] The protein, mineral and amino acid content was analyzed at PT Saraswanti Indo Genetech Laboratory (Bogor, Indonesia). Milling was conducted at the Indonesian Institute of Sciences, Physics Research Center, followed by the particle size analysis (PSA) at PT Cipta Mikro Material (Bogor, Indonesia). Fourier-transform infrared spectrometer (FTIR) and scanning electron microscopy (SEM) analyses were performed at the Forensic Laboratory Center of Indonesian National Police in Jakarta, Indonesia.

The analysis was conducted using liquid chromatography–mass spectrometry (LC-MS) (Shimadzu Corporation, Kyoto, Japan), ultra-performance liquid chromatography (UPLC) (ACQUITY Premier System; Waters Corporation, Milford, USA) and high-performance liquid chromatography (HPLC) (Waters Corporation). The eluent for LC-MS consisted of solution A (100 mM ammonium formate in water) and solution B (acetonitrile:water:formic acid, v:v:v = 95:5:0.3). The eluent for HPLC was ethanethiol. The retention times are presented in Table 1.

## Sample preparation

The LC-MS, UPLC and HPLC analyses required the sample to be hydrolyzed with 6N HCl 200 µL and 40 µL

Table 1. Amino acid content of *Stolephorus* sp. powder

| Parameter | Value [mg/kg] | Retention time [min] | Method |
|---|---|---|---|
| L-cysteine | 9,596.42 | 4.17–4.87 | 18-12-38/MU/SMMSIG (LC-MS) |
| L-methionine | 7,232.12 | 3.50–4.20 | 18-12-38/MU/SMMSIG (LC-MS) |
| L-serine | 32,648.37 | 5.95–6.65 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-glutamic acid | 64,598.39 | 5.31–6.01 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-phenylalanine | 3,6112.3 | 2.83–3.53 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-isoleucine | 28,542.91 | 3.32–4.02 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-valine | 33,534.56 | 3.86–4.56 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-alanine | 33,978.35 | 5.19–5.89 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-arginine | 45,869.46 | 9.24–9.94 | 18-5-17/MU/SMM-SIG (UPLC) |
| Glycine | 41,542.57 | 5.72–6.42 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-lysine | 40,155.18 | 8.49–9.19 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-aspartic acid | 39,802.95 | 5.60–6.30 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-leucine | 52,350.32 | 3.09–3.79 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-tyrosine | 26,236.71 | 3.79–4.49 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-proline | 24,326.38 | 3.57–4.27 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-threonine | 34,417.33 | 5.24–5.94 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-histidine | 20,571.09 | 8.42–9.12 | 18-5-17/MU/SMM-SIG (UPLC) |
| L-tryptophan | 4,899.71 | 2.97–3.67 | 18-5-63/MU/SMM-SIG (HPLC) |

LC-MS – liquid chromatography–mass spectrometry; UPLC – ultra-performance liquid chromatography; HPLC – high-performance liquid chromatography.

phenol, and dried in the oven at 112–116°C for 20–24 h. The excess HCl was wiped off and the tubes were subjected to vacuum drying. Subsequently, the derivatization of amino acids was conducted using an AccQ-Fluor Reagent Kit (Waters Corporation), followed by the separation of amino acids. Atomic absorption spectrophotometry (AAS) required the sample to be diluted with HCl and directly injected into the atomic absorption spectrophotometer.

## Protein, mineral and amino acid content analysis

The protein, mineral and amino acid content was analyzed. The total protein content of the sample was determined using a semi-automated device (behrotest® Steam Distiller type S2; behr Labor-Technik GmbH, Düsseldorf, Germany) that operates based on the Kjeldahl method.[11] The automatic addition of NaOH and $H_2O$ enables the steam distiller to identify the nitrogen content. The atomic absorption spectrophotometer (AA-6800; Shimadzu Corporation) was used to evaluate the mineral composition of the sample. This flame photometry tool determines the concentration of specific metals. The amino acid profile was analyzed using a mass spectrometer (ACQUITY Premier System; Waters Corporation).

## Particle size analysis

A top-down approach to reduce the particle size was performed in a manner similar to that described in the referenced study, with a modification in the milling time.[9] The *Stolephorus* sp. powder was subjected to high-energy milling for 8, 12 and 24 h, respectively. Zirconia balls were used, with a weight ratio of 1:5 between the powder and the balls. The rotational speed in the dry grinding mode was set at 100 rpm. Before and after milling, the particle size of *Stolephorus* sp. powder was measured using a particle size analyzer (Delsa™ Nano C; Beckman Coulter, Fullerton, USA). The dynamic light-scattering instrument required sample dilution, and fluctuations in scattered laser light, resulting from Brownian motion, were interpreted as the particle size.[12]

## Fourier-transform infrared spectrometry

The functional groups were identified using a FTIR (Alpha II; Bruker, Billerica, USA). The examination was performed on pellet samples of potassium bromide (KBr) (99.99%) mixed with the *Stolephorus* sp. powder. The FTIR spectrum was collected at a resolution of 4 $cm^{-1}$ in the transmission mode (400–4,000 $cm^{-1}$).

## Scanning electron microscopy–energy dispersive spectroscopy

Scanning electron microscopy–energy dispersive spectroscopy (SEM-EDS) (FlexSEM 1000; Hitachi Ltd., Tokyo, Japan) was used to determine the elemental composition of the material. For this examination, the samples were coated with gold. The tool was set at 10.00 kV, with a working distance of 9.8 mm and a pressure of 80.00 Pa.

## Statistical analysis

One-way analysis of variance (ANOVA) was used to examine the calcium/phosphorus (Ca/P) ratio between different milling time groups. The data was obtained from 3 repetition tests and is presented as mean ± standard deviation ($M \pm SD$). The analysis was performed using the IBM SPSS Statistics for Windows software, v. 25.0 (IBM Corp., Armonk, USA). The level of significance was set at $p < 0.05$.

# Results

The average particle size of *Stolephorus* sp. powder after blending and sieving was 17.41 ±1.80 µm. The protein content was 64.5%, and the mineral evaluation identified the presence of sodium, calcium and magnesium at concentrations of 7,420 mg/kg, 28,912 mg/kg and 1,924 mg/kg, respectively. The amino acid content of *Stolephorus* sp. powder is shown in Table 1.

Three *Stolephorus* sp. powders produced at various milling times demonstrated significantly different colors and degrees of smoothness (Fig. 1).

The test was followed by particle size measurement. The conversion of particle size from 17.41 ±1.8 μm to 463.3 ±105.7 nm (8-h milling), 1,365.4 ±161.3 nm (12-h milling) and 789.3 ±170.7 nm (24-h milling) was observed. The complete results, including the mean particle size, *SD* and polydispersity index are presented in Table 2.

The smallest mean particle size was observed in the 8-h milling sample, but the lowest polydispersity index was noted in the 12-h sample. The 24-h milling sample showed a particle size of 789.3 ±170.7 nm, with a polydispersity index of nearly 0.7 (0.763), thereby indicating the suitability of the measurement technique.

The organic and inorganic compounds present in the samples were identified through the use of the FTIR. The FTIR graphs (Fig. 2) indicated that the 3 samples produced at different milling times created a similar pattern. In addition, the graphs recorded more than 5 peaks of transmittance, which were identified as functional group compounds (Table 3).

The SEM-EDS was performed to describe the elemental content of the samples. The results of the SEM-EDS analysis are presented in Fig. 3 and Table 4.

The SEM-EDS showed the presence of significant and minor elements in the samples. Carbon, nitrogen and oxygen dominated the results, followed by calcium and phosphorus. The Ca/P ratio is a useful indicator of the presence of hydroxyapatite (HAp). The HAp reference stoichiometry was 1.67. The 24-h milling sample exhibited the Ca/P ratio most closely aligned with the reference value. However, one-way ANOVA showed no statistically significant difference between the groups ($p \geq 0.05$).

The SEM-EDS images (Fig. 4) were captured with a magnification range between 40 μm and 200 μm. The images facilitate the evaluation of particle distribution patterns and particle shapes. The scanning images of the *Stolephorus* sp. powder at 3 milling times showed that the milling time affected the particle distribution pattern. An extended milling time resulted in smoother particle distribution. Moreover, the images demonstrate that milling resulted in the formation of amorphous particles with a bumpy interface.

The changes in protein and mineral composition resulting from the milling process are shown in Table 5.

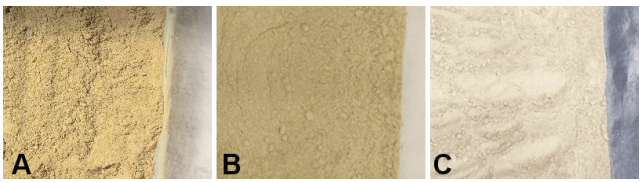


**Fig. 1.** *Stolephorus* sp. powders obtained at 3 milling times

A. Smooth powder with a dark color (8 h); B. More smooth powder with a darker color (12 h); C. Most smooth powder with the lightest color (12 h).

**Table 2.** Particle size of *Stolephorus* sp. powder at 3 milling times

| Milling time | Particle size [nm] *M* ±*SD* | Polydispersity index |
|---|---|---|
| 8 h | 463.3 ±105.7 | 1.245 |
| 12 h | 1,365.4 ±161.3 | 0.394 |
| 24 h | 789.3 ±170.7 | 0.763 |

*M* – mean; *SD* – standard deviation.

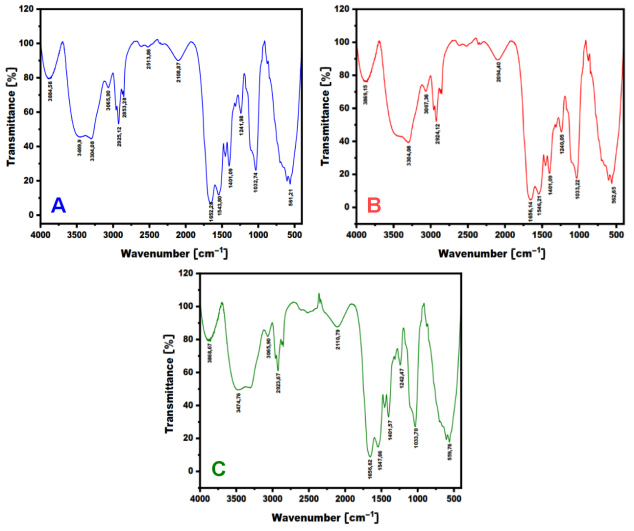


**Fig. 2.** Fourier-transform infrared spectrometer (FTIR) transmittance graphs of *Stolephorus* sp. powder at 3 milling times

A. 8 h; B. 12 h; C. 24 h.

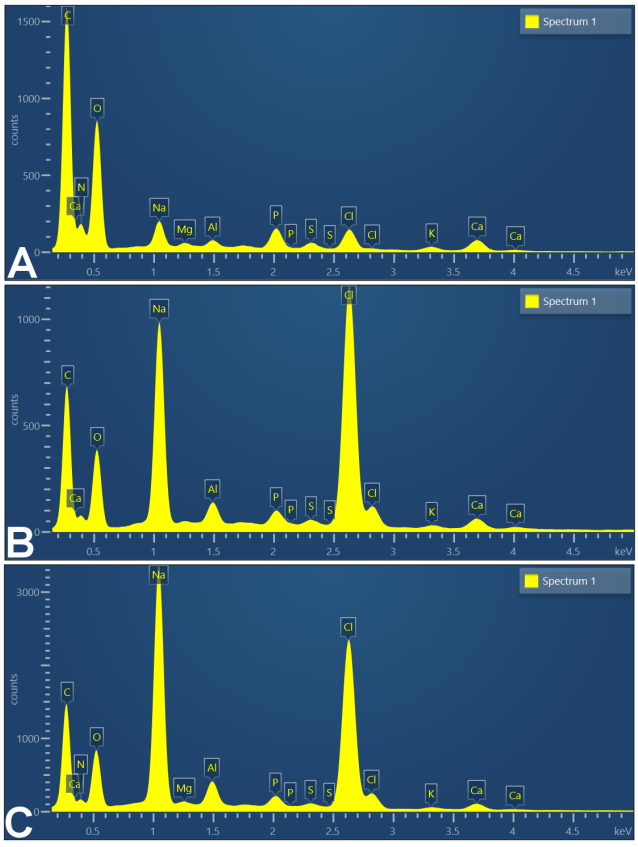


**Fig. 3.** Elemental spectrum of *Stolephorus* sp. powder at 3 milling times

A. 8 h; B. 12 h; C. 24 h.

**Table 3.** Fourier-transform infrared spectrometer (FTIR) transmittance peaks of *Stolephorus* sp. powder at 3 milling times

| Functional group | Wavenumber [$cm^{-1}$] | | |
|---|---|---|---|
| | 8 h | 12 h | 24 h |
| O–H | 3,884.58 | 3,869.15 | 3,868.67 |
| O–H | 3,469.9 | 3,304.08 | 3,474.76 |
| O–H | 3,304.08 | – | – |
| N–H | 3,065.90 | 3,067.36 | 3,065.90 |
| $CH_2$ | 2,925.12 | 2,924.12 | 2,923.67 |
| $CH_2$ | 2,853.28 | – | – |
| O–H | 2,513.86 | – | – |
| C≡C | 2,108.87 | 2,094.40 | 2,110.79 |
| C=O | 1,652.28 | 1,656.4 | 1,656.62 |
| N–H and C–N | 1,543.80 | 1,546.21 | 1,547.66 |
| $CH_3$ and $CH_2$ | 1,401.09 | 1,401.09 | 1,401.7 |
| C–N and N–H | 1,241.98 | 1,240.05 | 1,242.57 |
| P–O | 1,032.74 | 1,033.22 | 1,033.70 |
| $PO_4^{3-}$ | 561.21 | 562.65 | 559.76 |

**Table 4.** Elemental analysis of *Stolephorus* sp. powder at 3 milling times

| Element | Weight [%] | | |
|---|---|---|---|
| | 8 h *M ±SD* | 12 h *M ±SD* | 24 h *M ±SD* |
| C | 0.90 ±0.19 | 1.22 ±0.08 | 0.81 ±0.04 |
| N | 1.35 ±0.01 | 1.53 ±0.01 | 0.89 ±0.17 |
| O | 0.54 ±0.10 | 0.59 ±0.18 | 0.37 ±0.11 |
| Na | 0.16 ±0.09 | 0.17 ±0.08 | 0.13 ±0.09 |
| Mg | 0.06 ±0.01 | 0.07 ±0.01 | 0.05 ±0.01 |
| Al | 0.07 ±0.01 | 0.10 ±0.02 | 0.07 ±0.02 |
| P | 0.12 ±0.02 | 0.13 ±0.04 | 0.09 ±0.03 |
| S | 0.09 ±0.01 | 0.10 ±0.02 | 0.07 ±0.02 |
| Cl | 0.23 ±0.12 | 0.33 ±0.23 | 0.20 ±0.14 |
| K | 0.12 ±0.01 | 0.14 ±0.03 | 0.09 ±0.02 |
| Ca | 0.20 ±0.03 | 0.23 ±0.06 | 0.15 ±0.05 |
| Ca/P ratio | 1.68 ±0.09 | 1.82 ±0.16 | 1.72 ±0.05 |

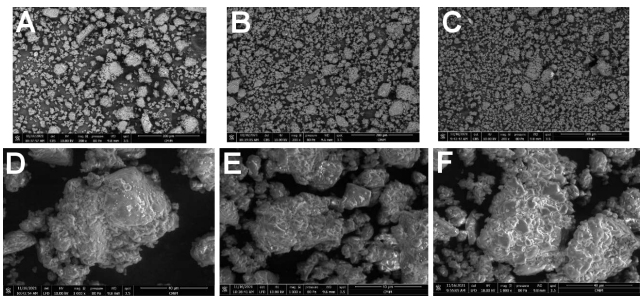


**Fig. 4.** Scanning electron microscopy–energy dispersive spectroscopy (SEM-EDS) images of *Stolephorus* sp. powder at 3 milling times

A. 8 h (×200 magnification); B. 12 h (×200 magnification); C. 24 h (×200 magnification); D. 8 h (×1,000 magnification); E. 12 h (×1,000 magnification); F. 24 h (×1,000 magnification).

**Table 5.** Comparison of protein and mineral composition of *Stolephorus* sp. powder before and after milling

| Substance | Initial (microscale) | 8 h | 12 h | 24 h |
|---|---|---|---|---|
| Protein [%] | 64.50 | 61.31 | 59.72 | 54.11 |
| Sodium [mg/kg] | 7,420 | 7,382 | 7,115 | 6,956 |
| Calcium [mg/kg] | 28,912 | 28,876 | 28,751 | 27,318 |
| Magnesium [mg/kg] | 1,924 | 1,918 | 1,877 | 1,843 |

## Discussion

The examination identified 18 amino acids and demonstrated high protein and mineral scores (Table 1). The results surpassed the findings of the previous study[13] due to a different sample preparation method. The previous study used fresh fish as a sample, whereas this study used the dried fish powder. This type of research has never been published before, as previous research has focused on the reduction of the size of fish bones, not the entire fish. In addition, this study used a different type of fish (snakehead) and employed calcination in a furnace prior to ball milling.[9] The previous study achieved a reduction in size to 38.9445 nm.[9] However, this technique is unsuitable for use with whole fish, because the protein is damaged by the calcination process. In this research, the FTIR test revealed the presence of proteins and organic compounds across the 8-, 12- and 24-h milling results, indicating no significant difference in pattern.

The amino acid content of the samples is beneficial for cell maintenance and repair, which is crucial for dental pulp tissue. Dental pulp tissue is surrounded by hard tissue, making it more susceptible to cell death when injured.[14] Studies on the roles of amino acids in cells are widespread in the medical field, but they are less prevalent in dentistry. Previous studies have demonstrated the importance of amino acids, such as valine, in the proliferation and maintenance of hematopoietic stem cells (HSCs). Human HSCs are unable to proliferate in valine-depleted conditions.[15] Another study described threonine as a nutritional modulator that influences the immune system via the mitogen-activated protein kinase and the target of the rapamycin signal pathway.[16] Methionine functions as a reactive oxygen species scavenger and a crucial player in the oxidative stress response.[17] Aspartate reduces doxorubicin toxicity in *Streptomyces cerevisiae* by providing carbon to the tricarboxylic acid cycle, and the addition of aspartate increases cell survival by promoting mitochondrial activity. The same effect can be achieved by the addition of asparagine, glutamate, glutamine, alanine, serine, lysine, and methionine.[18] Based on these studies, it can be stated that the *Stolephorus* sp. powder, a source of protein and amino acids, has great potential to promote cell repair.

Calcium ions were detected at a high level in the sample. These ions are established as second messengers in many cellular activities, whereby they regulate intracellular signals. A previous study demonstrated that calcium is beneficial in cell recruitment and regeneration.[19] These ions upregulate the expression of multiple cytokines in progenitor cells and have an influence on the extracellular signal of the calcium-sensing receptor. Receptor activation affects the chemotactic response of mesenchymal stem cells (MSCs) to calcium.[19] Calcium escalation has also been reported to cause cell proliferation and enhance the expression of osteopontin, inducing matrix mineralization of MSCs.[20] According to these findings, it can be posited that the *Stolephorus* sp. powder, as a natural calcium source, may promote bone and dentin regeneration.

These findings elucidate the results of various studies on *Stolephorus* sp. The daily intake of *Stolephorus* sp. has been demonstrated to increase the number of osteocytes and the mandibular alveolar bone density in Wistar rats.[5] Another study has shown that the application of *Stolephorus* sp. extract on dental pulp perforation increased the number of odontoblasts. Odontoblasts are responsible for the formation of dentin.[6]

The delivery of active pharmaceutical ingredients (APIs) is an important area of research. Particle size reduction has an impact on accelerating this process. Particle size reduction treatment increases the surface area, as well as the solubility and bioavailability of APIs.[21,22] Nanoscale particles are attained by a top-down or bottom-up approach.[23,24] The nanoscale range is 1–1,000 nm,[25] although some experts restrict it to <100 nm.

This study employed the top-down approach using high-energy ball milling. High-energy ball milling is a technique in which a powdered sample and grinding balls are placed in a container and subjected to a rotational motion. Two categories were identified within the process: mechanical alloying (MA); and mechanical disordering (MD). In MA, the elements of the powder are fractured and rewelded within a high-energy ball mill. In MD, the crystalline structure undergoes a transition to an amorphous state without altering its elemental composition.[26] Zirconia grinding balls were chosen due to their high density, hardness and toughness.

The results of this experiment demonstrated that the ball-milling process reduced the particle size of *Stolephorus* sp. powder from the microscale to the nanoscale. The duration of milling has an impact on the particle size and the polydispersity index. Although the PSA results indicated that 8 h of milling resulted in the smallest particle dimensions (463.3 ±105.7 nm), the SEM image of the powder milled for 8 h showed the roughest size distribution of particles. The 24-h milling powder occupied the second place (789.3 ±170.7 nm) and displayed the most uniform size distribution of particles in the SEM image. In addition to the average particle size, the PSA showed the polydispersity index of the samples. The polydispersity index ranges between 0.0 (monodisperse particle size) and 1.0 (multidisperse particle size).[27] The measurement of the 12-h milling powder showed the most significant particles among the remaining samples (1,365.4 nm), yet exhibited the lowest polydispersity index. The PSA tools used in the study operated based on the dynamic light scattering technique. A polydispersity index exceeding 0.7 indicates a broad size distribution of the sample.[28]

The SEM images showed that the particle size reduction process induced agglomeration (Fig. 4). Agglomeration can be attributed to the adhesion of particles due to the presence of weak forces, which ultimately results in the formation of a cluster of particles. Previous studies that used dry milling also documented this phenomenon.[29,30] Agglomeration obscured the PSA results. Thus, the findings should be confirmed by another method.[31]

The EDS analysis identified the presence of major elements (carbon, oxygen and nitrogen), known as protein composers.[32] This finding is also corroborated by the FTIR results, which revealed the presence of hydroxyl, N–H and $CH_2$ functional groups within the same spectral range as those reported in the previous studies.[33,34] Moreover, the other functional groups ($CH_3$, C–N and N–H), known as protein composers, were detected in areas that have been previously confirmed by other studies.[35,36]

The EDS examination revealed the presence of calcium and phosphorus, which originated from the scales and bones of *Stolephorus* sp.[37,38] The FTIR results showed asymmetrical stretching and P–O bands, which aligned with the findings of the previous studies.[33–35] The Ca/P ratios obtained from the elemental examination were compared to the HAp stoichiometry (1.67). According to multiple comparisons in the statistical analysis using one-way ANOVA, 24-h milling powder was found to be almost stoichiometric in nature. However, no significant difference was observed between the 3 groups. Hydroxyapatite is used in bone and dentin regeneration studies due to its chemical similarities with natural bones.[39,40] The compound facilitates osteoinduction, osteoconduction, osseointegration, remineralization, and dental pulp repair.[41–43]

The FTIR results for the *Stolephorus* sp. powder at different milling times showed an insignificant difference in transmittance and wavenumber. Therefore, the milling time did not influence the alteration of the functional group compounds. The peaks observed in the wavenumbers of 8-, 12- and 24-h milling samples, as illustrated in the wavenumber area of 3,869.15–3,304.08 $cm^{-1}$, indicate the presence of functional groups of O–H compounds, and are indicative of the presence of hydroxyl functional groups in fish protein. On the other hand, the wavenumbers of 3,065.90 $cm^{-1}$ and 3,067.36 $cm^{-1}$ show the vibration of the N–H compound group. Furthermore, the wavenumber area between 2,925.12 $cm^{-1}$ and 2,853.28 $cm^{-1}$ represents the functional group of the $CH_2$

compound. The wavenumber area between 2,110.79 $cm^{-1}$ and 2,108.87 $cm^{-1}$ indicates the stretching of the functional groups of the C≡C compounds. With regard to the wavenumber areas of 1,656.62 $cm^{-1}$, 1,656.14 $cm^{-1}$ and 1,652.28 $cm^{-1}$, they show sharp and prominent peaks, representing the functional groups of the C=O compounds derived from fish protein. Previous studies have also revealed that the FTIR spectrum of protein present in fish exhibits peaks at 3,304–3,315 $cm^{-1}$, 2,922–2,940 $cm^{-1}$, 1,600–1,700 $cm^{-1}$, 1,550–1,600 $cm^{-1}$, and 1,220–1,320 $cm^{-1}$.[33,34] The results of this study are consistent with the results of the synthesis that has been carried out.[33,34] Furthermore, the area of 1,401.57–1,401.09 $cm^{-1}$ represents the functional groups ($CH_3$ and $CH_2$) that constitute proteins.[36] The wavenumbers of 1,242.57 $cm^{-1}$, 1,240.05 $cm^{-1}$ and 1,241.98 $cm^{-1}$ show the protein's functional groups of C–N and N–H constituents.[35] The 3 powders also showed strong absorption peaks at approx. 1,032.74 $cm^{-1}$, 1,033.22 $cm^{-1}$ and 1,033.70 $cm^{-1}$, which were marked by the asymmetric stretching of the phosphate group (P–O).[35] The more visible peaks or bands at 561.21 $cm^{-1}$, 562.65 $cm^{-1}$ and 559.76 $cm^{-1}$ correspond to $PO_4^{3-}$ residues from fish samples.[33,42]

Although the milling process can change the protein and mineral content, its influence was not significant in this study (Table 5). The high-energy milling process generates heat, which can alter the material's physicochemical properties. They can be influenced by speed, milling time, moisture content, pre-treatment process, type of ball mill pot, ball size, proportion of pot filling, and other factors.[44,45] Therefore, to minimize the reduction in protein content, the milling procedure was performed with a pot volume of 55%, at a low speed and with a large ball size. According to previous studies, this process can decrease the size of protein particles, reduce the temperature rise and maintain the physicochemical properties of the product.[44–47] Thus, in our study, there was a decrease in the protein and mineral content from baseline to the longest grinding time. However, the observed difference was not significant. This finding is consistent with the results of previous research.[46,47]

# Conclusions

The findings of this study lay the foundation for future research using *Stolephorus* sp. as an inducer of dental pulp cell repair. Further research will continue to develop vital pulp therapy using nanosized materials derived from *Stolephorus* sp. In addition, in vivo tests will be conducted to evaluate the formation and quality of reparative dentin induced in pulp perforation. The high mineral and protein content can be maintained throughout the particle size reduction process. A reduction in the particle size enhances bioavailability and accelerates the recuperation time.

## 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

Anastasia Elsa Prahasti https://orcid.org/0000-0003-2709-5081
Tamara Yuanita https://orcid.org/0000-0002-5320-7772
Retno Pudji Rahayu https://orcid.org/0000-0003-4429-3653


### References


1. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT; International Natural Product Sciences Taskforce. Natural products in drug discovery: Advances and opportunities. *Nat Rev Drug Discov*. 2021;20(3):200–216. doi:10.1038/s41573-020-00114-z
2. Prahasti AE, Yuanita T. Utilization of anchovy in dentistry. *Malaysian J Med Health Sci*. 2021;17(Supp6):87–90. https://repository.unair.ac.id/123692. Accessed August 16, 2023.
3. Rauf FH, Tangke U, Namsa D. Dinamika Populasi Ikan Teri (*Stolephorus* sp) yang di Daratkan di Pasar Higienis Kota Ternate. *J Biol Sains Teknol*. 2019;1(1):1–9. doi:10.52046/biosainstek.v1i01.206.1-9
4. Safyanti, Andrafikar. Efficacy of micronutrient supplementation from local food of bada fish (*Stolephorus insularis*) on the status of anemia among adolescents in Padang city. *KnE Life Sci*. 2019;2019:111–115. doi:10.18502/kls.v4i15.5744
5. Astrina I, Primasari A, Keumalasari D. Anchovy intake to increase alveolar bone density of the mandibles in white wistar rats (*Rattus norvegicus*). *IOSR J Dent Med Sci*. 2018;17(3):28–33. doi:10.9790/0853-1703092833
6. Effendi MC, Alrista R, Taufiq A, Susanto H. Effects of anchovy (*Stolephorus* sp.) hydroxyapatite on the number of odontoblast cells for reparative dentine stimulation on *Rattus norvegicus* teeth. *AIP Conf Proc*. 2021:1–7. doi:10.1063/5.0052863
7. MacDonell R, Hamrick MW, Isales CM. Protein/amino-acid modulation of bone cell function. *Bonekey Rep*. 2016;5:827. doi:10.1038/bonekey.2016.58
8. Sari RP, Revianti S, Nugroho HK. Application direct pulp capping *Stolephorus insularis* cream to dentin Reparatif. In: Kalanjati VP, ed. *The Future of Anatomy. 6th Asia Pacific International Congress of Anatomy*. 2011:134–137. https://dspace.hangtuah.ac.id/xmlui/bitstream/handle/dx/1033/APICA%202011.pdf?sequence=3&isAllowed=y. Accessed August 16, 2023.
9. Muryati M, Hariani PL, Said M. Preparation and characterization nanoparticle calcium oxide from snakehead fish bone using ball milling method. *Indones J Fundamental Appl Chem*. 2019;4(3):111–115. doi:10.24845/ijfac.v4.i3.111
10. Hoshyar N, Gray S, Han H, Bao G. The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction. *Nanomedicine (Lond)*. 2016;11(6):673–692. doi:10.2217/nnm.16.5
11. Mæhre HK, Dalheim L, Edvinsen GK, Elvevoll EO, Jensen IJ. Protein determination – method matters. *Foods*. 2018;7(1):5. doi:10.3390/foods7010005
12. Zhang H, Baeyens J, Kang Q. Measuring suspended particle size with high accuracy. *Int J Petrochem Sci Eng*. 2017;2(6):2–7. doi:10.15406/ipcse.2017.02.00058
13. Sankar TV, Anandan R, Mathew S, et al. Chemical composition and nutritional value of Anchovy (*Stolephorus commersonii*) caught from Kerala coast, India. *Eur J Exp Biol*. 2013;3(1):85–89.

14. Rombouts C, Giraud T, Jeanneau C, About I. Pulp vascularization during tooth development, regeneration, and therapy. *J Dent Res*. 2017;96(2):137–144. doi:10.1177/0022034516671688
15. Taya Y, Ota Y, Wilkinson AC, et al. Depleting dietary valine permits nonmyeloablative mouse hematopoietic stem cell transplantation. *Science*. 2016;354(6316):1152–1155. doi:10.1126/science.aag3145
16. Tang Q, Tan P, Ma N, Ma X. Physiological functions of threonine in animals: Beyond nutrition metabolism. *Nutrients*. 2021;13(8):2592. doi:10.3390/nu13082592
17. Campbell K, Vowinckel J, Keller MA, Ralser M. Methionine metabolism alters oxidative stress resistance via the pentose phosphate pathway. *Antioxid Redox Signal*. 2016;24(10):543–547. doi:10.1089/ars.2015.6516
18. Dornfeld K, Madden M, Skildum A, Wallace KB. Aspartate facilitates mitochondrial function, growth arrest and survival during doxorubicin exposure. *Cell Cycle*. 2015;14(20):3282–3291. doi:10.1080/15384101.2015.1087619
19. Aquino-Martínez R, Angelo AP, Pujol FV. Calcium-containing scaffolds induce bone regeneration by regulating mesenchymal stem cell differentiation and migration. *Stem Cell Res Ther*. 2017;8(1):265. doi:10.1186/s13287-017-0713-0
20. Lee MN, Hwang HS, Oh SH, et al. Elevated extracellular calcium ions promote proliferation and migration of mesenchymal stem cells via increasing osteopontin expression. *Exp Mol Med*. 2018;50(11):1–16. doi:10.1038/s12276-018-0170-6
21. Khadka P, Ro J, Kim H, et al. Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. *Asian J Pharm Sci*. 2014;9(6):304–316. doi:10.1016/j.ajps.2014.05.005
22. Afifi SA, Hassan MA, Abdelhameed AS, Elkhodairy KA. Nanosuspension: An emerging trend for bioavailability enhancement of etodolac. *Int J Polym Sci*. 2015;2015:11–15. doi:10.1155/2015/938594
23. Salazar J, Müller RH, Möschwitzer JP. Combinative particle size reduction technologies for the production of drug nanocrystals. *J Pharm (Cairo)*. 2014;2014:265754. doi:10.1155/2014/265754
24. Mageswari A, Srinivasan R, Subramanian P, Ramesh N, Gothandam KM. Nanomaterials: Classification, Biological Synthesis and Characterization. In: Ranjan S, Dasgupta N, Lichtfouse E, eds. *Nanoscience in Food and Agriculture 3*. 3rd ed. 2016:31–71. doi:10.1007/978-3-319-48009-1
25. Rogers B, Adams J, Pennathur S. Introduction to Miniaturization. In: *Nanotechnology*. 3rd ed. Boca Raton, FL: CRC Press; 2015:51–76. doi:10.1201/b17424-6
26. Sherif El-Eskandarany M, Al-Hazza A, Al-Hajji LA, et al. Mechanical milling: A superior nanotechnological tool for fabrication of nanocrystalline and nanocomposite materials. *Nanomaterials*. 2021;11(10):2484. doi:10.3390/nano11102484
27. Danaei M, Dehghankhold M, Ataei S, et al. Impact of particle size and polydisperity index on the clinical applications of lipidic nanocarrier system. *Pharmaceutics*. 2018;10(2):57. doi:10.3390/pharmaceutics10020057
28. Stetefeld J, McKenna SA, Patel TR. Dynamic light scattering: A practical guide and applications in biomedical sciences. *Biophys Rev*. 2016;8(4):409–427. doi:10.1007/s12551-016-0218-6
29. Guzzo PL, Marinho de Barros FB, Soares BR, Santos JB. Evaluation of particle size reduction and agglomeration in dry grinding of natural quartz in a planetary ball mill. *Powder Technol*. 2020;368:149–159. doi:10.1016/j.powtec.2020.04.052
30. Blanc N, Mayer-Laigle C, Frank X, Radjai F, Delenne JY. Evolution of grinding energy and particle size during dry ball-milling of silica sand. *Powder Technol*. 2020;376:661–667. doi:10.1016/j.powtec.2020.08.048
31. Gosens I, Post JA, de la Fonteyne LJJ, et al. Impact of agglomeration state of nano- and submicron sized gold particles on pulmonary inflammation. *Part Fibre Toxicol*. 2010;7(1):37. doi:10.1186/1743-8977-7-37
32. van Goudoever JB, Vlaardingerbroek H, van den Akker CH, de Groof F, van der Schoor SRD. Amino acids and proteins. *World Rev Nutr Diet*. 2014;110:49–63. doi:10.1159/000358458
33. Zhang Y, Tu D, Shen Q, Dai Z. Fish scale valorization by hydrothermal pretreatment followed by enzymatic hydrolysis for gelatin hydrolysate production. *Molecules*. 2019;24(16):2998. doi:10.3390/molecules24162998
34. Riaz T, Zeeshan R, Zarif F, et al. FTIR analysis of natural and synthetic collagen. *Appl Spectrosc Rev*. 2018;53(9):703–746. doi:10.1080/05704928.2018.1426595
35. Sasmal P, Begam H. Extraction of type-I collagen from sea fish and synthesis of hap/collagen composite. *Procedia Mater Sci*. 2014;5:1136–1140. doi:10.1016/j.mspro.2014.07.408
36. Hernández-Martínez M, Gallardo-Velázquez T, Osorio-Revilla G, Almaraz-Abarca N, Ponce-Mendoza A, Vásquez-Murrieta MS. Prediction of total fat, fatty acid composition and nutritional parameters in fish fillets using MID-FTIR spectroscopy and chemometrics. *LWT – Food Sci Technol*. 2013;52(1):12–20. doi:10.1016/j.lwt.2013.01.001
37. Amaral Corrêa TH, França Holanda JN. Fish bone as a source of raw material for synthesis of calcium phosphate. *Mater Res*. 2019;22(Suppl 1):e20190486. doi:10.1590/1980-5373-MR-2019-0486
38. Granito RN, Renno ACM, Yamamura H, de Almeida MC, Ruiz PLM, Ribeiro DA. Hydroxyapatite from fish for bone tissue engineering: A promising approach. *Int J Mol Cell Med*. 2018;7(2):80–90. doi:10.22088/IJMCM.BUMS.7.2.80
39. Kattimani VS, Kondaka S, Lingamaneni KP. Hydroxyapatite – past, present, and future in bone regeneration. *Bone Tissue Regener Insights*. 2016;7:S36138. doi:10.4137/btri.s36138
40. Von Euw S, Wang Y, Laurent G, et al. Bone mineral: New insights into its chemical composition. *Sci Rep*. 2019;9(1):1–11. doi:10.1038/s41598-019-44620-6
41. Sathiyavimal S, Vasantharaj S, LewisOscar F, Selvaraj R, Brindhadevi K, Pugazhendhi A. Natural organic and inorganic – hydroxyapatite biopolymer composite for biomedical applications. *Prog Org Coat*. 2020;147:105858. doi:10.1016/j.porgcoat.2020.105858
42. Shi P, Liu M, Fan F, Yu C, Lu W, Du M. Characterization of natural hydroxyapatite originated from fish bone and its biocompatibility with osteoblasts. *Mater Sci Eng C Mater Biol Appl*. 2018;90:706–712. doi:10.1016/j.msec.2018.04.026
43. Okamoto M, Matsumoto S, Sugiyama A, et al. Performance of a biodegradable composite with hydroxyapatite as a scaffold in pulp tissue repair. *Polymers (Basel)*. 2020;12(4):937. doi:10.3390/POLYM12040937
44. Schmidt R, Martin Scholze H, Stolle A. Temperature progression in a mixer ball mill. *Int J Ind Chem*. 2016;7(2):181–186. doi:10.1007/s40090-016-0078-8
45. Tharanon W, Peerapattana J. The effects of ball mill processing on the physicochemical properties of glutinous rice starch. *Thai J Pharm Sci*. 2020;44(2):91–8. https://www.thaiscience.info/Journals/Article/TJPS/10996283.pdf. Accessed August 19, 2024.
46. Wang Z, Chang S, Li Y, et al. Effects of ball milling treatment on physicochemical properties and digestibility of Pacific oyster (*Crassostrea gigas*) protein powder. *Food Sci Nutr*. 2018;6(6):1582–1590. doi:10.1002/fsn3.705
47. Chen X, Guo M, Sang Y, Sun J. Effect of ball-milling treatment on the structure, physicochemical properties and allergenicity of proteins from oyster (*Crassostrea gigas*). *LWT*. 2022;166:113803. doi:10.1016/j.lwt.2022.113803

Original paper

# Effectiveness of white tea-mediated silver nanoparticles as an intracanal irrigant against *Enterococcus faecalis*: An in vitro study

Lakshimi Lakshmanan[1,A–D], Deepa Gurunathan[1,A,E,F], Rajeshkumar Shanmugam[2,A,E,F]

[1] Department of Pediatric and Preventive Dentistry, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India
[2] Nanobiomedicine Lab, Centre for Global Health Research, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, 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*. 2024;61(4):593–598

**Address for correspondence**
Deepa Gurunathan
E-mail: drgdeepa@yahoo.co.in

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on April 10, 2021
Reviewed on October 13, 2021
Accepted on November 2, 2021

Published online on August 16, 2024

## Abstract

**Background.** The probability of a positive outcome of root canal therapy is substantially higher if the infection is eradicated successfully before the obturation of the root canal system. Irrigation is an essential aspect of root canal debridement, as it enables more thorough cleaning than is possible with root canal instrumentation alone. To overcome the side effects of chemical irrigants, there has been a search for herbal medicines as substitutes.

**Objectives.** The aim of the present study was to explore the antimicrobial efficacy of white tea-mediated silver nanoparticles (AgNPs) formulated as an intracanal irrigant against *Enterococcus faecalis*, and to compare it with the efficacy of chlorhexidine and sodium hypochlorite irrigants.

**Material and methods.** The experimental groups were as follows: group I – white tea-mediated AgNPs; group II – 2% chlorhexidine; and group III – 2.5% sodium hypochlorite. The characterization of AgNPs was performed using ultraviolet-visible (UV-Vis) spectroscopy and transmission electron microscopy (TEM) analysis. *Enterococcus faecalis* was inoculated onto Mueller–Hinton agar plates. The disks impregnated with irrigants were placed on the inoculated plates and incubated aerobically at 37°C for 24 h. Then, the growth inhibition zones were measured. Statistical analysis was performed using the one-way analysis of variance (ANOVA) and the post hoc tests.

**Results.** A concentration of 50 µL of white tea-mediated AgNPs exhibited the greatest zone of inhibition (32 ±2 mm), followed by 2% chlorhexidine (25 ±1 mm) and 2.5% sodium hypochlorite (23 ±3 mm).

**Conclusions.** White tea-mediated AgNPs showed promising results in the elimination of *E. faecalis*, being superior to chlorhexidine and sodium hypochlorite irrigants.

**Keywords:** herbal, silver nanoparticles, root canal therapy, white tea, irrigant

**Cite as**
Lakshmanan L, Gurunathan D, Shanmugam R. Effectiveness of white tea-mediated silver nanoparticles as an intracanal irrigant against *Enterococcus faecalis*: An in vitro study. *Dent Med Probl.* 2024;61(4):593–598. doi:10.17219/dmp/143545

**DOI**
10.17219/dmp/143545

# Introduction

The success of endodontic treatment relies on the thorough disinfection of the root canal system. The role of microorganisms in the pathogenesis of pulp-periapical lesions is well-established in the literature.[1] *Enterococcus faecalis*, in particular, has gained special attention, as it is one of the dominant microbes isolated from failed root canals. It is more resistant to endodontic therapy due to its ability to penetrate dentinal tubules and its resistance to intracanal medicaments.[2,3]

Studies have shown that necrotic tissues, the microbial by-products remaining within dentinal tubules, canal ramifications, and resorption pits that are resistant to mechanical instruments result in persistent periradicular inflammation.[4] Therefore, chemo-mechanical preparation, which involves various instrumentation techniques, irrigation protocols and intracanal medicaments, is proposed to prevent the further entry of microorganisms from an infected root canal and to create conditions conducive to tissue healing.[4,5]

Intracanal irrigation complements the instrumentation technique by expediting the removal of necrotic tissues and microbes from the root canal. Various studies have reported that portions of root canal walls remain undebrided during mechanical instrumentation.[6–8] Hence, irrigating agents with strong antimicrobial activity are essential adjuncts to instrument preparation, thereby aiding in the removal of pulp remnants and residual microorganisms from intricate root canals.[5] Chlorhexidine and sodium hypochlorite have been utilized globally for irrigating both permanent and primary root canals. Recently, there has been increased interest in herbal agents to replace chemical irrigants due to their limitations, such as tissue toxicity, allergic reactions, the discoloration of clothes, and unpleasant odor and taste. Several herbal irrigants with antimicrobial and therapeutic effects have been suggested for use as endodontic irrigants.[9–11]

White tea, which is extracted in an unfermented form from the leaves of the tea plant *Camellia sinensis*, possesses strong antioxidant properties in addition to its medicinal properties due to high levels of polyphenols.[12] Nanoparticles (NPs) have also been studied in the field of endodontics to reduce *E. faecalis* adherence to dentine and to reinforce root canal disinfection.[13]

The purpose of the current study was to explore the antimicrobial efficacy of white tea-mediated silver nanoparticles (AgNPs) formulated as an intracanal irrigant against *E. faecalis*, and to compare it with the efficacy of chlorhexidine and sodium hypochlorite irrigants.

# Material and methods

The approval for the current in vitro study was obtained from the Ethics Committee at the Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India (SRB/SDC/PEDO-1803/20/05). The experimental groups were as follows: group I – white tea-mediated AgNPs; group II – 2% chlorhexidine; and group III – 2.5% sodium hypochlorite.

## Preparation of the white tea extract and the incorporation of AgNPs

Fresh white tea leaves (Ketley Gold Marketing, Assam, India) in the amount of 10 g were boiled for 15 min in 50 mL of distilled water. The obtained plant extract was filtered through sterile filter paper. In another container, 0.0169 g of silver nitrate ($AgNO_3$) powder was added to 50 mL of distilled water and stirred continuously to obtain a $AgNO_3$ solution. The plant extract and the $AgNO_3$ solution were thoroughly mixed, using an electronic magnetic stirrer for 8 h. The mixture was then placed in test tubes and centrifuged for 15 min at 8,000 rpm.

## UV-Vis spectroscopy and TEM analysis

The formation of AgNPs through the reduction of $AgNO_3$ was monitored at different time intervals up to 72 h, between 250 and 700 nm. A transmission electron microscope (TEM) was used to examine the surface morphology.

## Isolation of microorganisms

A total of 100 µL of *E. faecalis* suspension taken from the prepared cultures was inoculated onto culture plates, 130 mm in diameter, with the previously set layers of Mueller–Hinton agar.

## Antimicrobial activity

Using a sterile cork borer (HiMedia Laboratories, Mumbai, India), 3 wells (approx. 0.5 mm in diameter) were cut into the Mueller–Hinton agar plates. Using a micropipette, approx. 25 µL, 50 µL and 100 µL of the white tea-mediated AgNP solution was transferred into the wells, and then impregnated in sterilized 3-millimeter blank disks. The disks were placed on the Mueller–Hinton agar plates with gentle pressure against the agar surface to ensure uniform contact. The plates were incubated at 37°C for 24 h, and the antimicrobial efficacy was then evaluated.

To compare antimicrobial activity among the groups included in the study, the concentration of white tea-mediated AgNPs which exhibited the greatest zone of inhibition (group I) was further compared with 2% chlorhexidine (Asep-RC; Anabond Stedman Pharma Research, Chennai, India) (group II) and 2.5% sodium hypochlorite (Prime Dental Products, Thane, India) (group III) in Petri dishes containing 3 wells of a 0.5-millimeter diameter, using the same method as described earlier.

For the antimicrobial activity assessment, the diameter of the inhibition zone around the disks was measured using a ruler to the nearest whole millimeter. To ensure reliability, all experiments were replicated 3 times, and the average of the 3 measurements was recorded as the final value.

### Statistical analysis

The recorded data was analyzed using the IBM SPSS Statistics for Windows, v. 23.0 (IBM Corp., Armonk, USA). Statistical significance was determined with the one-way analysis of variance (ANOVA) and the post hoc tests, with a $p$-value set at 0.05.

## Results

### Incorporation of AgNPs into the white tea extract

The addition of the white tea extract to the $AgNO_3$ solution initially resulted in a dark brown color, which then faded to lighter brown after continued magnetic stirring.

### UV-Vis spectroscopy and TEM analysis

The UV-Vis spectroscopy results are shown in Fig. 1. The formation of AgNPs was observed between 250 and 700 nm. White tea-mediated AgNPs exhibited a peak between 420 and 460 nm, confirming their synthesis. The TEM analysis revealed that AgNPs were uniformly distributed, spherical in shape, and had a particle size ranging from 30 nm to 35 nm (Fig. 2).

### Antimicrobial activity

The antimicrobial activity of white tea-mediated AgNPs at 3 different concentrations was assessed by measuring the zone of inhibition against *E. faecalis* in a disk diffusion test (Table 1). The 50 µL concentration of white tea-mediated AgNPs showed the largest zone of inhibition.

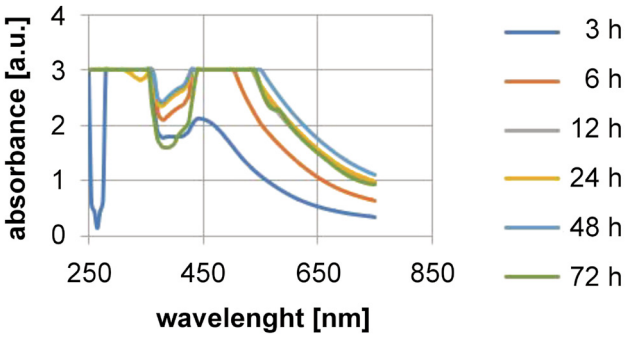


Fig. 1. Ultraviolet-visible (UV-Vis) spectroscopy values

Comparative tests with 2% chlorhexidine and 2.5% sodium hypochlorite indicated that white tea-mediated AgNPs exhibited a larger zone of inhibition than both chlorhexidine and sodium hypochlorite irrigants (Table 2).

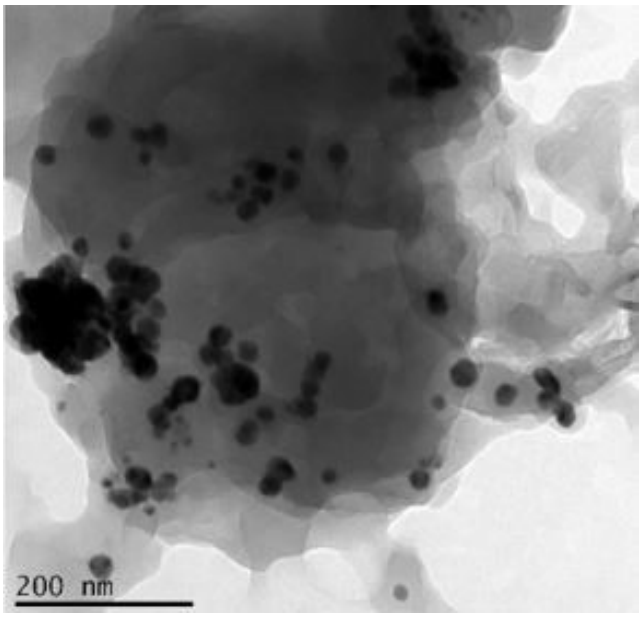


Fig. 2. Transmission electron microscopy (TEM) analysis

Table 1. Antimicrobial activity of various concentrations of a white tea-mediated silver nanoparticle (AgNP) solution

| Concentration [µL] | Zone of inhibition against *Enterococcus faecalis* [mm] |
|---|---|
| 25 | 15 ±1 |
| 50 | 32 ±2* |
| 100 | 14 ±1 |

Data presented as mean ± standard deviation (*M* ±*SD*).
* statistically significant difference with regard to other concentrations ($p < 0.05$).

Table 2. Comparison of the antimicrobial efficacy of a white tea-mediated silver nanoparticle (AgNP) solution against chlorhexidine and sodium hypochlorite solutions

| Irrigant | Zone of inhibition against *Enterococcus faecalis* [mm] |
|---|---|
| White tea-mediated AgNPs (50 µL) | 32 ±2* |
| 2% chlorhexidine | 25 ±1 |
| 2.5% sodium hypochlorite | 23 ±3 |

Data presented as *M* ±*SD*.
* statistically significant difference with regard to other irrigants ($p < 0.05$).

## Discussion

The main goal of any treatment is to provide the optimal benefit to the patient with minimal harm and discomfort. In the case of primary teeth, thorough mechanical preparation is not desirable due to the presence of thin dentinal walls. Consequently, root canal irrigants play a significant

role in the debridement of the root canal system.[4,14] Sodium hypochlorite, chlorhexidine gluconate, ethylenediaminetetraacetic acid (EDTA), citric acid, hydrogen peroxide, and other root canal irrigating solutions have been suggested for use in clinical practice for primary teeth.[15] Chemical irrigants, however, cannot be used too frequently in primary teeth due to their lower safety profile. To address the side effects of chemical irrigants, there has been a search for herbal alternatives. With the rise of antibiotic-resistant strains, it has become essential to explore herbal medicines with strong antimicrobial properties to improve the outcome of biomechanical procedures.[16,17]

*Enterococcus faecalis* was chosen as the test organism, as this facultative Gram-positive bacterium is commonly associated with secondary infections, with a prevalence rate of 24–77%, and it rapidly colonizes dentinal tubules.[2,18]

The agar diffusion test was used in this study, as it allows the direct comparison of test materials against microorganisms, indicating whether the material has the potential to kill bacteria in the local environment. It is also one of the most widely used methods for determining the antimicrobial activity of endodontic irrigants.[19,20]

Sodium hypochlorite, with concentrations ranging from 0.5% to 6%, has been recommended for root canal irrigation. It is inexpensive, readily available, has a long shelf life, and excellent tissue-dissolving and antibacterial properties.[21] The chlorine (Cl) in sodium hypochlorite exerts an antimicrobial effect by inhibiting bacterial enzymes, leading to the irreversible oxidation of crucial bacterial enzyme sulfhydryl groups. The strong pH of sodium hypochlorite contributes to its antimicrobial properties by disrupting cytoplasmic membrane integrity, altering cellular metabolism and causing the degradation of phospholipids.[22] However, when sodium hypochlorite is accidentally injected past the root apex, it can cause tissue reactions, such as pain, swelling, necrosis, and hemorrhage.[10]

Chlorhexidine gluconate is a cationic bisbiguanide that acts by adsorbing onto the cell walls of microorganisms and inducing the leakage of intracellular components.[23] Chlorhexidine has a bacteriostatic effect at low concentrations, allowing the leaching of small-molecular-weight compounds from microorganisms. At higher concentrations, chlorhexidine has bactericidal activity due to cytoplasmic precipitation and/or coagulation, likely mediated by protein cross-linking.[24] Clinical trials have reported severe reactions, including postoperative pain, following the extrusion of the chlorhexidine irrigant into periapical tissues.[25]

A mild form of tea, white tea, is produced from the young leaves of the tea plant *C. sinensis*. It retains high catechin concentrations found in fresh tea leaves.[12] White tea is processed the least, so it contains the most polyphenols, which are not oxidized or lost during processing. It has more potent antioxidants than green tea, and has been shown to be even more beneficial. The high content of epigallocatechin gallate is primarily responsible for its strong antioxidant properties.[26,27]

Silver nanoparticles bind to and penetrate the cell walls of both Gram-positive and Gram-negative bacteria, disrupting cell function by releasing Ag ions. As a result, they are used for fighting drug-resistant microorganisms and inhibiting biofilm formation.[13] In dental practice, AgNPs are incorporated into the bonding agents and restorative materials to prevent biofilm formation and reduce caries. Nanoparticles are used in endodontics to minimize *E. faecalis* adherence to dentine, remove biofilm and improve root canal disinfection.[28,29] This study aimed to evaluate the efficacy of white tea and AgNPs in combination against *E. faecalis*.

The activity of AgNPs in the prepared white tea solution was confirmed by visual observation, UV-Vis spectroscopy, and TEM analysis. Free electrons in metal NPs produce a surface plasmon resonance (SPR) absorption band due to the mutual vibration of electrons in resonance with light waves.[28] Peaks in UV-Vis spectroscopy confirmed the SPR characteristics of AgNPs in the prepared white tea solution. The particle size was confirmed by TEM micrographs as 30–35 nm. According to Shrestha et al., NP characteristics, such as contact time, concentration, particle size, and surface charge, affect their antimicrobial activity.[30] Afkhami et al. reported that irrigation with 100 ppm AgNPs had the same antimicrobial potency as 2.5% sodium hypochlorite irrigation.[31]

In the present study, the zones of inhibition of bacterial growth achieved with white tea-mediated AgNPs were greater than those obtained with other irrigants for *E. faecalis*. This indicates that this herbal irrigant has higher efficacy against *E. faecalis* as compared to sodium hypochlorite and chlorhexidine. This result contrasts with the findings of Jose et al.[9] and Saxena et al.,[10] who found that sodium hypochlorite was more effective against *E. faecalis* than the herbal irrigants tested. Sodium hypochlorite showed a mean inhibition zone of 23 mm for *E. faecalis*, which is consistent with the analysis by Jose et al.[9] Other researchers have reported similar findings regarding the use of chlorhexidine as an endodontic irrigant.[24,25] It has been proven that 2% chlorhexidine effectively removes the smear layer in 15 s and enriches the dentinal surface with chlorhexidine digluconate.[32] Baca et al. reported 100% biofilm inhibition when using 2% chlorhexidine against *E. faecalis*.[33] According to Garcia et al., 0.12% chlorhexidine has a significant inhibitory effect on the activity of dentinal proteolytic enzymes.[34] In terms of inhibition zone, 2% chlorhexidine was found to be comparable to sodium hypochlorite.

According to the findings of this study, white tea-mediated AgNPs had the greatest antibacterial potency and the optimal inhibition ability. Its biocompatible antioxidant nature renders it an effective endodontic irrigant.

A major limitation of this in vitro study is that the results cannot be fully transferred to clinical scenarios, since the efficacy of extracts may be affected by additional factors, such as staining, substantivity, as well as action

against other caries-causing microorganisms, which were not evaluated.

Advanced methods, such as the ETEST®, which combines the disk diffusion and agar dilution methods, or bioautography techniques, could be used in future research to explore these natural extracts further in the search for new antimicrobial irrigants.

Most previous research has focused on evaluating herbal irrigants in vitro; thus, detailed in vivo research is needed to assess the long-term stability of AgNPs, the susceptibility and toxicity of such irrigants, as well as their capacity to act against various other microorganisms.

# Conclusions

Within the limitations of the present study, white tea-mediated AgNPs showed promising results in the elimination of *E. faecalis*, demonstrating superior efficacy in comparison with chlorhexidine and sodium hypochlorite irrigants. Further in vitro and clinical studies are required to evaluate their efficacy, biocompatibility and safety before they can be conclusively recommended as a root canal irrigant.

## Ethics approval and consent to participate

The approval for the current in vitro study was obtained from the Ethics Committee at the Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India (SRB/SDC/PEDO-1803/20/05).

## Data availability

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

## Consent for publication

Not applicable.

### ORCID iDs

Lakshimi Lakshmanan https://orcid.org/0000-0002-9815-0837
Deepa Gurunathan https://orcid.org/0000-0002-6014-946X
Rajeshkumar Shanmugam https://orcid.org/0000-0001-7059-8894

### References

1. Möller AJ. Microbiological examination of root canals and periapical tissues of human teeth. Methodological studies. *Odontol Tidskr*. 1966;74(5):1–380. PMID:5335186.
2. Rôças IN, Siqueira JF Jr., Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. *J Endod*. 2004;30(5):315–320. doi:10.1097/00004770-200405000-00004
3. Siqueira JF Jr., Rôças IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2004;97(1):85–94. doi:10.1016/s1079-2104(03)00353-6
4. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. *Oral Surg Oral Med Oral Pathol*. 1983;55(3):307–312. doi:10.1016/0030-4220(83)90333-x
5. Kaur R, Singh R, Sethi K, Garg S, Miglani S, Vats S. Irrigating solutions in pediatric dentistry: Literature review and update. *J Adv Med Dent Sci*. 2014;2(2):104–115. https://jamdsr.com/pdf2b/IrrigatingSolutionsinPediatricDentistryLiteratureReviewandUpdate.pdf. Accessed April 1, 2021.
6. Kandaswamy D, Venkateshbabu N. Root canal irrigants. *J Conserv Dent*. 2010;13(4):256–264. doi:10.4103/0972-0707.73378
7. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. *J Endod*. 2006,32(2):93–98. doi:10.1016/j.joen.2005.10.049
8. Siqueira JF Jr., Rôças IN, Favieri A, Lima KC. Chemomechanical reduction of the bacterial population in the root canal after instrumentation and irrigation with 1%, 2.5%, and 5.25% sodium hypochlorite. *J Endod*. 2000;26(6):331–334. doi:10.1097/00004770-200006000-00006
9. Jose J, Shoba K, ShibuAman, NithyaTomy, Sheena, Christie. Comparative evaluation of antimicrobial activity of green tea extract, garlic extract, neem leaf extract and sodium hypochlorite as root canal irrigants against *E. faecalis* and *C. albicans* – an in vitro study. *Int J Curr Microbiol App Sci*. 2015;4(10):384–391. https://www.ijcmas.com/vol-4-10/Jerin%20Jose,%20et%20al.pdf. Accessed April 1, 2021.
10. Saxena D, Saha SG, Saha MK, Dubey S, Khatri M. An in vitro evaluation of antimicrobial activity of five herbal extracts and comparison of their activity with 2.5% sodium hypochlorite against *Enterococcus faecalis*. *Indian J Dent Res*. 2015;26(5):524–527. doi:10.4103/0970-9290.172080
11. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. *Microbes Infect*. 1999;1(2):125–129. doi:10.1016/s1286-4579(99)80003-3
12. Shanmugapriya K, Nityanandi D, Saravana PS. Evaluation of antioxidant, anti-inflammatory and antimicrobial activities of two different extracts of *Camellia sinensis* (L.) O. Kuntze. *Int J Drug Dev Res*. 2013;5(3):155–168. https://www.itmedicalteam.pl/articles/evaluation-of-antioxidant-antiinflammatory-and-antimicrobial-activities-of-two-different-extracts-of-camellia-sinensis-l-o-kuntze.pdf. Accessed April 1, 2021.
13. Rodrigues CT, De Andrade FB, De Vasconcelos LR, et al. Antibacterial properties of silver nanoparticles as a root canal irrigant against *Enterococcus faecalis* biofilm and infected dentinal tubules. *Int Endod J*. 2018;51(8):901–911. doi:10.1111/iej.12904
14. Wang YL, Chang HH, Kuo CI, et al. A study on the root canal morphology of primary molars by high-resolution computed tomography. *J Dent Sci*. 2013;8(3):321–327. doi:10.1016/j.jds.2013.04.002
15. Sumikawa DA, Marshall GW, Gee L, Marshall SJ. Microstructure of primary tooth dentin. *Pediatr Dent*. 1999;21(7):439–444. PMID:10633518.
16. Agnihotri A, Arora R, Sharma U, Sood P. Herbal irrigants in primary teeth: A step toward green dentistry based on the wisdom of past. *J Indian Assoc Public Health Dent*. 2020;18(4):279–284. doi:10.4103/jiaphd.jiaphd_109_20
17. Jolly M, Singh N, Rathore M, Tandon S, Banerjee M. Propolis and commonly used intracanal irrigants: Comparative evaluation of antimicrobial potential. *J Clin Pediatr Dent*. 2013;37(3):243–249. doi:10.17796/jcpd.37.3.3434221kn05tl376
18. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. *Int Endod J*. 1998;31(1):1–7. PMID:9823122.
19. Figueiredo de Almeida Gomes BP, Vianna ME, Sena NT, Zaia AA, Randi Ferraz CC, De Souza Filho FJ. In vitro evaluation of the antimicrobial activity of calcium hydroxide combined with chlorhexidine gel used as intracanal medicament. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;102(4):544–550. doi:10.1016/j.tripleo.2006.04.010
20. Abdulkader A, Duguid R, Saunders EM. The antimicrobial activity of endodontic sealers to anaerobic bacteria. *Int Endod J*. 1996;29(4):280–283. doi:10.1111/j.1365-2591.1996.tb01382.x
21. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. *J Endod*. 1992;18(12):605–612. doi:10.1016/S0099-2399(06)81331-2

22. Zand V, Lotfi M, Rahimi S, Mokhtari H, Kazemi A, Sakhamanesh V. A comparative scanning electron microscopic investigation of the smear layer after the use of sodium hypochlorite gel and solution forms as root canal irrigants. *J Endod*. 2010;36(7):1234–1237. doi:10.1016/j.joen.2010.02.033
23. Mohammadi Z. Chlorhexidine gluconate, its properties and applications in endodontics. *Iran Endod J*. 2008;2(4):113–125. PMID:24265633.
24. Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. *J Endod*. 1994;20(6):276–278. doi:10.1016/s0099-2399(06)80815-0
25. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. *Int Endod J*. 2001;34(6):424–428. doi:10.1046/j.1365-2591.2001.00410.x
26. Sajilata MG, Bajaj PR, Singhal RS. Tea polyphenols as nutraceuticals. *Compr Rev Food Sci Food Saf*. 2008;7(3):229–254. doi:10.1111/j.1541-4337.2008.00043.x
27. Almajano MP, Carbo R, López Jiménez J, Gordon MH. Antioxidant and antimicrobial activities of tea infusions. *Food Chem*. 2008;108(1):55–63. doi:10.1016/j.foodchem.2007.10.040
28. Sreenivasagan S, Subramanian AK, Rajeshkumar S. Assessment of antimicrobial effects and cytotoxic effect of green mediated silver nanoparticles and its coating onto mini-implants. *Ann Phytomed*. 2020;9(1):207–212. doi:10.21276/ap.2020.9.1.27
29. Samiei M, Farjami A, Dizaj SM, Lotfipour F. Nanoparticles for antimicrobial purposes in endodontics: A systematic review of in vitro studies. *Mater Sci Eng C Mater Biol Appl*. 2016;58:1269–1278. doi:10.1016/j.msec.2015.08.070
30. Shrestha A, Shi Z, Neoh KG, Kishen A. Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. *J Endod*. 2010;36(6):1030–1035. doi:10.1016/j.joen.2010.02.008
31. Afkhami F, Pourhashemi SJ, Sadegh M, Salehi Y, Kharazzi Fard MJ. Antibiofilm efficacy of silver nanoparticles as a vehicle for calcium hydroxide medicament against *Enterococcus faecalis*. *J Dent*. 2015;43(12):1573–1579. doi:10.1016/j.jdent.2015.08.012
32. Lapinska B, Klimek L, Sokolowski J, Lukomska-Szymanska M. Dentine surface morphology after chlorhexidine application – SEM study. *Polymers (Basel)*. 2018;10(8):905. doi:10.3390/polym10080905
33. Baca P, Junco P, Arias-Moliz MT, González-Rodríguez MP, Ferrer-Luque CM. Residual and antimicrobial activity of final irrigation protocols on *Enterococcus faecalis* biofilm in dentin. *J Endod*. 2011;37(3):363–366. doi:10.1016/j.joen.2010.11.036
34. Garcia MB, Carrilho MR, Nör JE, et al. Chlorhexidine inhibits the proteolytic activity of root and coronal carious dentin in vitro. *Caries Res*. 2009;43(2):92–96. doi:10.1159/000209340

Original paper

# Effect of glide path preparation on the apical debris extrusion for three single-file systems

Kübra Yeşildal Yeter[A,C–F], Betul Gunes[A,C,E,F], Emine Zeynep Bal[A,B,F]

Department of Endodontics, Faculty of Dentistry, Eskişehir Osmangazi University, Eskişehir, 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*. 2024;61(4):599–604

**Address for correspondence**
Kübra Yeşildal Yeter
E-mail: kubrayesildal@gmail.com

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
The present study was presented at the Turkish Dental Association (TDA) 25th International Dental Congress, which was held on September 4–7, 2019, in Istanbul, Turkey.
The authors would like to thank Dr. Yasin Altay from the Faculty of Agriculture of Eskişehir Osmangazi University, Turkey, for statistical analysis.

Received on December 8, 2020
Reviewed on May 5, 2021
Accepted on May 7, 2021

Published online on August 30, 2024

## Abstract

**Background.** The dentinal debris produced during root canal preparation can be extruded through the apical foramen, which may cause undesired consequences, such as the postoperative inflammation of periapical tissues.

**Objectives.** The aim of the present in vitro study was to evaluate the effect of glide path preparation on apical debris extrusion for the Reciproc®, WaveOne Gold® and One Curve file systems.

**Material and methods.** A total of 72 mandibular incisors were divided into 6 groups according to the nickel-titanium (NiTi) system used and glide path preparation: group Rp – Reciproc; group Rp-0 – Reciproc after glide path preparation; group WG – WaveOne Gold; group WG-0 – WaveOne Gold after glide path preparation; group OC – One Curve; and group OC-0 – One Curve after glide path preparation. The pre- and post-instrumentation weight of Eppendorf tubes was measured. The pre-weight was subtracted from the post-weight to calculate the amount of apically extruded debris. The data was analyzed using the one-way analysis of variance (ANOVA) with the statistical program PASW Statistics for Windows, v.18.0.

**Results.** No statistically significant differences were found between the single-NiTi file systems with different kinematics in terms of apical debris extrusion, with or without glide path preparation ($p > 0.05$).

**Conclusions.** The amount of apically extruded debris was not affected by the kinematics of different single-NiTi file systems. Moreover, creating a glide path had no effect on the apically extruded debris in straight root canals.

**Keywords:** root canal preparation, dental instruments, root canal therapy, endodontics

**Cite as**
Yeşildal Yeter K, Gunes B, Bal EZ. Effect of glide path preparation on the apical debris extrusion for three single-file systems. *Dent Med Probl.* 2024;61(4):599–604. doi:10.17219/dmp/136464

**DOI**
10.17219/dmp/136464

## Introduction

Postoperative symptomatic apical periodontitis may occur as an undesirable consequence of root canal treatment, and the patient can feel mild to severe pain while chewing.[1] It has been previously mentioned that the prevalence of pain 24–48 h after root canal treatment is 40–65%.[2] Postoperative pain depends on many factors, such as the existence of preoperative pain, occlusal trauma, and injuries due to the chemical, mechanical and bacterial irritation of periapical tissues during root canal treatment.[3,4] During root canal preparation, the debris containing dentin chips, bacteria and necrotic tissue remnants can be extruded through the periapical area, which is assumed to be one of the major causes of post-treatment periapical inflammation.[5] Many factors, such as the instrument design, the number of instruments, the kinematics of the instrument system, the size of apical preparation, and the determination of the working length, can affect the amount of apically extruded debris.[6–9]

Reciprocating single-file nickel-titanium (NiTi) systems were introduced as an alternative to continuous rotational multiple-file NiTi systems. It has been claimed that this technique is associated with a safer root canal preparation procedure, decreased cost, reduced preparation time, and the prevention of cross-contamination risk.[10] According to a systematic review of studies on reciprocating and continuous rotation, the reciprocating motion is advantageous with regard to fracture resistance, but its effects in terms of shaping ability, dentinal defects and apical debris extrusion are controversial.[10]

Reciproc® (VDW, Munich, Germany) is the first reciprocating single-file NiTi system. This system is produced with a different NiTi alloy, called M-wire, which is subjected to a different thermal process. This process ensures increased flexibility and mechanical strength of the file.[11] The Reciproc file has 2 cutting edges and its cross-section is S-shaped.

The WaveOne® Gold system (Dentsply Maillefer, Ballaigues, Switzerland) has been marketed with the claim that its increased elasticity is due to the heat treatment of the NiTi alloy, which is called Gold-wire. The Gold-wire technology is a procedure of thermocycling, which involves heating and slowly cooling the file. This procedure results in the file changing to a gold color.[12] The file has a parallelogram cross-section with 2 cutting edges.

One Curve (Micro-Mega, Besançon, France) is a heat-treated rotary file that enables shaping the root canal with a single instrument. One Curve is manufactured from a C-wire NiTi alloy, which has a controlled memory property. The manufacturer claims that the pre-bending ability of the One Curve file enhances the process of shaping the root canal. The One Curve instrument has variable cross-sections along the blade.[13] To the best of our knowledge, there is one study that compares the effect of this rotational NiTi single-file system with the reciprocating movement on apical debris extrusion.[14]

A glide path is a smooth tunnel that extends from the orifice of the canal to the minor apical foramen.[15] Glide path preparation before the enlargement of the main root canal has been recommended to minimize the risk of shaping aberrations, file separation and taper lock.[16] A glide path can be formed with either rotary glide path files or hand files. Glide path preparation with manual K-files may be disadvantageous, as the procedure is more time-consuming and more difficult than creating a glide path with rotary NiTi file systems.[17] It has also been reported that using manual K-files to form a glide path can result in more postoperative pain.[18]

One G (Micro-Mega) is a single-file rotary glide path system. The tip diameter of the file is 0.14 mm and the taper is 0.03 throughout the shaft. One G has an asymmetrical cross-section with 3 cutting edges. The manufacturer claims that the design of the file provides a decreased screwing effect. Also, 3 different cutting edges with 3 different radii result in better debris elimination in the coronal direction.[19] A recent study showed that One G caused less debris extrusion than manual K-files.[20]

The aim of the present in vitro study was to evaluate the effect of single-file NiTi systems with different kinematics, alloys and designs on the apically extruded debris with or without rotary glide path preparation. The null hypothesis was that there would be no difference between the Reciproc, WaveOne Gold and One Curve files in terms of the amount of apically extruded debris, with or without glide path preparation.

## Material and methods

The procedure of the study was approved by the Ethics Committee at Eskişehir Osmangazi University, Turkey, with the reference number 25403353-050.99-E.110594. A total of 72 freshly extracted mandibular incisor teeth, removed for periodontal and prosthetic reasons, were selected for this study. The inclusion criteria for the specimens were that the teeth should have mature apices, no previous endodontic treatment, Vertucci I root canal configuration, and no visible cracks on the root surface. The extracted teeth were cleaned with a scaler to remove tissue remnants, and placed in 5% sodium hypochlorite (NaOCl) for 10 min for disinfection. The teeth were then washed with tap water and stored in distilled water until use. The length of all teeth was standardized to 18 mm by decoronation, and endodontic access cavities were prepared using diamond burs under water cooling. Eppendorf tubes were pre-weighed on an electronic scale with a precision of $10^{-5}$ g (Mettler-Toledo, Greifensee, Switzerland). Three consecutive measurements were obtained to calculate the average weight of the tubes.

## Debris collection

An experimental model was prepared for the evaluation of apical debris, as described by Myers and Montgomery.[21] The pre-weighed Eppendorf tubes were used for debris collection. Holes were created in the rubber stoppers of the vials, and the specimen was inserted through the rubber stopper and fixed with Teflon tape to prevent the leakage of the irrigation solution through the space between the rubber stopper and the specimen. A 27-gauge needle was inserted into the Eppendorf tube through the rubber stopper to equalize air pressure. The entire experimental setup was obscured with a rubber dam sheet to prevent the operator from seeing the apex (Fig. 1).

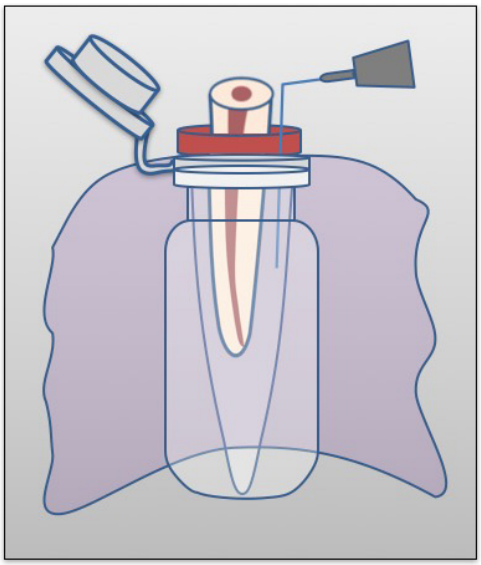

Fig. 1. Illustration of the experimental setup that was used to collect the apically extruded debris

## Root canal instrumentation

The specimens were randomly divided into 6 groups ($n$ = 12) according to root canal preparation. A #10 K-file (VDW) was inserted into the root canal until it was visible at the apical foramen, and the working length was established by subtracting 1 mm from this length. Teeth with apical patency of no more than ISO #10 were included in this study. The groups were as follows:

– group Rp – root canals were prepared with a Reciproc file (VDW), with a #25 tip diameter and 0.08 taper;
– group Rp-O – glide path preparation was done with a One G file (Micro-Mega), and then root canals were prepared with the Reciproc file;
– group WG – root canals were prepared with a WaveOne Gold primary file (Dentsply Maillefer), with a #25 tip diameter and 0.07 taper;
– group WG-O – glide path preparation was done with a One G file, and then root canals were prepared with the WaveOne Gold primary file;
– group OC – root canals were prepared with a One Curve file (Micro-Mega), with a #25 tip diameter and 0.06 taper; and
– group OC-O – glide path preparation was done with a One G file, and then root canals were prepared with the One Curve file.

The One Curve file was used with an in-and-out motion without pressure at a rotational speed of 300 rpm and a torque of 2.5 N·m, using a torque-controlled endodontic motor (X-Smart Plus; Dentsply Maillefer). A slow, in-and-out pecking motion was used for the Reciproc and WaveOne Gold files, according to the manufacturers' instructions. After several in-and-out movements, the files were removed and cleaned. The canal was irrigated with distilled water, and apical patency was confirmed using a #10 K-file. This procedure was repeated until the NiTi instrument reached the working length.

Distilled water in the amount of 8 mL was used as an irrigation solution with a syringe and a 29-gauge side-vented NaviTip™ irrigation needle (Ultradent Products, Inc., South Jordan, USA). Apical patency was assessed with a #10 K-file before final irrigation. Final irrigation was performed with 2 mL of distilled water. The root apex was washed with 0.5 mL of distilled water to collect the debris adhering to the external surface. During root canal preparation, the apically extruded debris was collected in the pre-weighed Eppendorf tubes. The tubes were stored in an incubator at 68°C for 5 days to allow the evaporation of distilled water. Three consecutive weight measurements were obtained, and the mean post-instrumentation weight was calculated for each tube. The amount of extruded debris was calculated by subtracting the pre-instrumentation weight from the post-instrumentation weight of the Eppendorf tubes (Fig. 2). A single operator performed all experimental procedures.

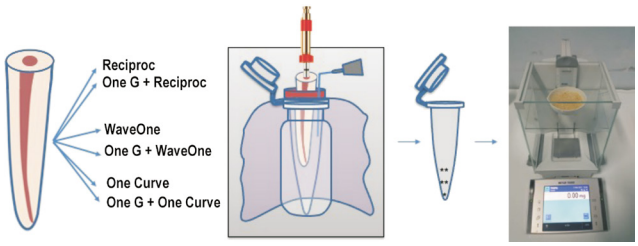


Fig. 2. Schematic presentation of the experimental procedures

## Statistical analysis

The study had a randomized plot design with the root canal preparation technique as an independent factor and 12 replicates. The one-way analysis of variance (ANOVA) was conducted to analyze apical debris extrusion. All statistical analyses were performed using the PASW Statistics for Windows, v. 18.0 (SPSS Inc., Chicago, USA), with a significance level of 5%.

The mathematical model of variance analysis (Equation 1):

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \quad (1)$$

where:
$Y_{ij}$ – the $j^{th}$ observation on the $i^{th}$ level of treatment;
$\mu$ – the common effect for the whole experiment;
$\alpha_i$ – the $i^{th}$ treatment fixed effect; and
$\varepsilon_{ij}$ – random error terms.

## Results

The minimum, maximum, mean, and standard deviation values with regard to the apically extruded debris for each experimental group are shown in Table 1. The box plots are presented in Fig. 3. No statistically significant differences were found among the experimental groups ($p > 0.05$). All procedures were associated with the extrusion of debris through the apical foramen.

Table 1. Amount of apically extruded debris [g] for all experimental groups

| Group | *n* | min | max | *M ±SD* |
|---|---|---|---|---|
| Rp | 12 | 0.000020 | 0.000580 | 0.000279 ±0.000182[a] |
| Rp-O | 12 | 0.000050 | 0.000440 | 0.000187 ±0.000114[a] |
| WG | 12 | 0.000090 | 0.000550 | 0.000268 ±0.000143[a] |
| WG-O | 12 | 0.000030 | 0.000610 | 0.000308 ±0.000180[a] |
| OC | 12 | 0.000060 | 0.000370 | 0.000201 ±0.000087[a] |
| OC-O | 12 | 0.000090 | 0.000470 | 0.000284 ±0.000119[a] |

Groups: Rp – Reciproc; Rp-O – Reciproc after glide path preparation; WG – WaveOne Gold; WG-O – WaveOne Gold after glide path preparation; OC – One Curve; and OC-O – One Curve after glide path preparation.
min – minimum; max – maximum; M – mean; SD – standard deviation.
The same superscript letters represent no statistically significant difference.

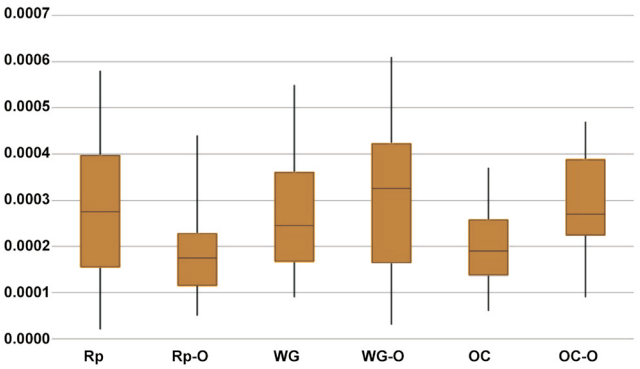


Fig. 3. Box plots showing the amount of extruded debris (the minimum, maximum, median, and standard deviation values) for all experimental groups

## Discussion

The aim of this in vitro study was to evaluate the amount of apically extruded debris during the preparation of root canals, using 3 different single-file systems with or without glide path preparation. According to the results of this study, no statistically significant differences were found among the experimental groups. The null hypothesis was accepted. It was impossible to prevent apical debris extrusion in any of the experimental groups.

The experimental setup described by Myers and Montgomery[21] was used for the collection of the apically extruded debris (Fig. 1). Since it is not possible to measure the amount of apically extruded debris under clinical conditions, in vitro studies are still conducted to quantify this parameter. It must be kept in mind that in this experimental setup, the absence of apical back pressure may negatively affect the amount of apically extruded debris.[22] Previously, floral foam has been used to simulate apical negative pressure and overcome the limitations of this setup.[23] However, floral foam may absorb the irrigation solution and debris, thus influencing the test results.[21,22] The apical preparation size is another factor that can affect the amount of apically extruded debris.[9] The size of the main file should be the same in all experimental groups for standardization. In this study, all single-file systems had the same apical size (#25) to ensure standardization. The volume and type of the irrigation solutions used in the preparation procedure might also affect the amount of apically extruded debris.[7] NaOCl crystals can remain in the collected debris after the evaporation procedure, which may affect the test results. In this study, distilled water was used for all irrigation procedures to evaluate solely the effect of the file type and glide path preparation. Also, the volume of the irrigation solution was kept the same for all experimental groups. It has been previously found that the determination of the working length as –1 mm from the apical foramen significantly decreases the amount of apically extruded debris.[21] Therefore, in this study, the working length was determined as –1 mm from the apical foramen.

Previous in vitro studies have examined whether there is a difference in the amount of apically extruded debris in the teeth prepared with either multiple-file or single-file systems.[22,24–26] Whereas some studies report that multiple-file systems cause more apically extruded debris, blaming the increased number of files,[24,26] others report that it is the single-file systems that cause more debris extrusion.[25,27] Also, in a recent systematic review, it was concluded that the design of the file is the most dominant factor affecting the apically extruded debris.[7] Studies comparing the effect of single-file reciprocating and full-sequence rotational systems have also reported different results.[22,28,29] Various types of cross-sections and the taper of the files, specimens with straight or curved canals, the design of irrigation needles, and the preparation protocols used in each study might be responsible for the different study results. According to a recent report on apical debris extrusion, the WaveOne Gold single-file reciprocating system causes a similar amount of debris extrusion as the Reciproc system

and less debris extrusion than the HyFlex™ EDM single-file rotational system.[29] This partially contrasts with our findings. Our results showed that there was no significant difference between the 2 single-file reciprocating systems and the single-file rotational system. This difference may result from the different designs of full-sequence rotational system files, such as taper and cross-section, and different study designs. To our knowledge, only one study has examined apical debris extrusion for the One Curve system; the previous study reported that the amount of apically extruded debris was greater for WaveOne Gold as compared to One Curve.[14] This difference with regard to the findings of the present study may result from the different study designs and operative effects.

Glide path preparation before the enlargement of the root canal has been recommended to reduce procedural errors.[16] Manual files and multiple-file rotary NiTi glide path systems have been used for glide path preparation. Single-file NiTi glide path systems were designed to reduce the number of files and the time spent on creating a glide path. According to our study, creating a glide path with the One G glide path file did not affect apical debris extrusion in any experimental group. Topçuoğlu et al. stated that creating a glide path before preparing root canals with reciprocating and rotational single files reduced the amount of apically extruded debris.[30] This result is not consistent with our results. A possible reason for this may be the different study design. In the present study, mandibular incisors were used, whereas Topçuoğlu et al. used mandibular molars with curved canals.[30]

Taking into account the construction of the experimental setup in the present study, it was more likely to mimic the natural position of mandibular teeth. Gravitational force will have different effects on the amount of irrigant and debris extrusion in mandibular and maxillary teeth. Also, the absence of apical back pressure might affect the amount of irrigant and debris extrusion. Therefore, clinicians should interpret the results of this study with caution. Within the limitations of this study, it can be concluded that creating a glide path before root canal preparation with single-file reciprocating or rotational NiTi systems did not change the amount of apically extruded debris in straight root canals. However, the possible advantages of creating a glide path are not limited to reducing apical debris extrusion. Other advantages, such as decreasing the risk of shaping aberrations, file separation and taper lock, should also be considered.

# Conclusions

The amount of apically extruded debris was not influenced by either reciprocating or full-sequence rotational single-file NiTi systems. Furthermore, creating a glide path did not affect the amount of apically extruded debris in straight root canals.

## Ethics approval and consent to participate

The procedure of the study was approved by the Ethics Committee at Eskişehir Osmangazi University, Turkey, with the reference number 25403353-050.99-E.110594.

## Data availability

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

## Consent for publication

Not applicable.

### ORCID iDs

Kübra Yeşildal Yeter https://orcid.org/0000-0002-7450-4119
Betul Gunes https://orcid.org/0000-0001-7070-3033
Emine Zeynep Bal https://orcid.org/0000-0002-3746-4920

### References

1. Gutmann JL, Baumgartner JC, Gluskin AH, Hartwell GR, Walton RE. Identify and define all diagnostic terms for periapical/periradicular health and disease states. *J Endod*. 2009;35(12):1658–1674. doi:10.1016/j.joen.2009.09.028
2. Pak JG, White SN. Pain prevalence and severity before, during, and after root canal treatment: A systematic review. *J Endod*. 2011;37(4):429–438. doi:10.1016/j.joen.2010.12.016
3. Caviedes-Bucheli J, Azuero-Holguin MM, Correa-Ortiz JA, et al. Effect of experimentally induced occlusal trauma on substance P expression in human dental pulp and periodontal ligament. *J Endod*. 2011;37(5):627–630. doi:10.1016/j.joen.2011.02.013
4. Glennon JP, Ng YL, Setchell DJ, Gulabivala K. Prevalence of and factors affecting postpreparation pain in patients undergoing two-visit root canal treatment. *Int Endod J*. 2004;37(1):29–37. doi:10.1111/j.1365-2591.2004.00748.x
5. Siqueira JF, Jr. Microbial causes of endodontic flare-ups. *Int Endod J*. 2003;36(7):453–463. doi:10.1046/j.1365-2591.2003.00671.x
6. Bürklein S, Schäfer E. Apically extruded debris with reciprocating single-file and full-sequence rotary instrumentation systems. *J Endod*. 2012;38(6):850–852. doi:10.1016/j.joen.2012.02.017
7. Caviedes-Bucheli J, Castellanos F, Vasquez N, Ulate E, Munoz HR. The influence of two reciprocating single-file and two rotary-file systems on the apical extrusion of debris and its biological relationship with symptomatic apical periodontitis. A systematic review and meta-analysis. *Int Endod J*. 2016;49(3):255–270. doi:10.1111/iej.12452
8. Koçak S, Koçak MM, Sağlam BC, Türker SA, Sağsen B, Er Ö. Apical extrusion of debris using self-adjusting file, reciprocating single-file, and 2 rotary instrumentation systems. *J Endod*. 2013;39(10):1278–1280. doi:10.1016/j.joen.2013.06.013
9. Tanalp J, Güngör T. Apical extrusion of debris: A literature review of an inherent occurrence during root canal treatment. *Int Endod J*. 2014;47(3):211–221. doi:10.1111/iej.12137
10. Ahn SY, Kim HC, Kim E. Kinematic effects of nickel-titanium instruments with reciprocating or continuous rotation motion: A systematic review of in vitro studies. *J Endod*. 2016;42(7):1009–1017. doi:10.1016/j.joen.2016.04.002
11. Pereira ES, Peixoto IF, Viana AC, et al. Physical and mechanical properties of a thermomechanically treated NiTi wire used in the manufacture of rotary endodontic instruments. *Int Endod J*. 2012;45(5):469–474. doi:10.1111/j.1365-2591.2011.01998.x
12. Elsaka SE, Elnaghy AM, Badr AE. Torsional and bending resistance of WaveOne Gold, Reciproc and Twisted File Adaptive instruments. *Int Endod J*. 2017;50(11):1077–1083. doi:10.1111/iej.12728

13. Micro-Mega. One Curve brochure. https://micro-mega.com/wp-content/uploads/2020/11/60301900-A_Flyer-One-Curve-Portfolio-EN_web.pdf. Accessed May 6, 2021.
14. Kumari H, Prasad SD, Sunil kumar C, Krishna NV, Sunil kumar S, Chandra Babu KS. Comparative evaluation of apical extrusion of debris during root canal preparation using two rotary systems – an in vitro study. *Acta Sci Dent Sci*. 2019;3(12):139–143. doi:10.31080/ASDS.2019.03.0715
15. West JD. The endodontic Glidepath: "Secret to rotary safety". *Dent Today*. 2010;29(9):86. PMID:20973422.
16. Berutti E, Cantatore G, Castellucci A, et al. Use of nickel-titanium rotary PathFile to create the glide path: Comparison with manual preflaring in simulated root canals. *J Endod*. 2009;35(3):408–412. doi:10.1016/j.joen.2008.11.021
17. Paleker F, Van der Vyver PJ. Glide path enlargement of mandibular molar canals by using K-files, the ProGlider file, and G-files: A comparative study of the preparation times. *J Endod*. 2017;43(4):609–612. doi:10.1016/j.joen.2016.11.025
18. Keskin C, Yilmaz ÖS, Inan U, Özdemir Ö. Postoperative pain after glide path preparation using manual, reciprocating and continuous rotary instruments: A randomized clinical trial. *Int Endod J*. 2019;52(5):579–587. doi:10.1111/iej.13053
19. Uslu G, Ozyurek T, Inan U. Comparison of Cyclic Fatigue Resistance of ProGlider and One G Glide Path Files. *J Endod*. 2016;42(10):1555-1558. doi:10.1016/j.joen.2016.07.012
20. Gunes B, Yesildal Yeter K. Effects of different glide path files on apical debris extrusion in curved root canals. *J Endod*. 2018;44(7):1191–1194. doi:10.1016/j.joen.2018.04.012
21. Myers GL, Montgomery S. A comparison of weights of debris extruded apically by conventional filing and Canal Master techniques. *J Endod*. 1991;17(6):275–279. doi:10.1016/S0099-2399(06)81866-2
22. Bürklein S, Benten S, Schäfer E. Quantitative evaluation of apically extruded debris with different single-file systems: Reciproc, F360 and OneShape versus Mtwo. *Int Endod J*. 2014;47(5):405–409. doi:10.1111/iej.12161
23. Altundasar E, Nagas E, Uyanik O, Serper A. Debris and irrigant extrusion potential of 2 rotary systems and irrigation needles. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011;112(4):e31–e35. doi:10.1016/j.tripleo.2011.03.044
24. De-Deus G, Neves A, Silva EJ, et al. Apically extruded dentin debris by reciprocating single-file and multi-file rotary system. *Clin Oral Investig*. 2015;19(2):357–361. doi:10.1007/s00784-014-1267-5
25. Küçükyilmaz E, Savas S, Saygili G, Uysal B. Assessment of apically extruded debris and irrigant produced by different nickel-titanium instrument systems. *Braz Oral Res*. 2015;29:1–6. doi:10.1590/1807-3107bor-2015.vol29.0002
26. Silva EJ, Carapiá MF, Lopes RM, et al. Comparison of apically extruded debris after large apical preparations by full-sequence rotary and single-file reciprocating systems. *Int Endod J*. 2016;49(7):700–705. doi:10.1111/iej.12503
27. Arslan D, Kustarci A. Efficacy of photon-initiated photoacoustic streaming on apically extruded debris with different preparation systems in curved canals. *Int Endod J*. 2018;51(Suppl 1):e65–e72. doi:10.1111/iej.12816
28. Pawar AM, Pawar M, Kfir A, Thakur B, Mutha P, Banga KS. Effect of glide path preparation on apical extrusion of debris in root canals instrumented with three single-file systems: An ex vivo comparative study. *J Conserv Dent*. 2017;20(2):110–114. doi:10.4103/0972-0707.212235
29. Elashiry MM, Saber SE, Elashry SH. Apical extrusion of debris after canal shaping with three single-file systems. *Niger J Clin Pract*. 2020;23(1):79–83. doi:10.4103/njcp.njcp_319_19
30. Topçuoğlu HS, Düzgün S, Akpek F, Topçuoğlu G, Aktı A. Influence of a glide path on apical extrusion of debris during canal preparation using single-file systems in curved canals. *Int Endod J*. 2016;49(6):599–603. doi:10.1111/iej.12484

Review

# Primary stability with osseodensification drilling of dental implants in the posterior maxilla region in humans: A systematic review

Alejandro Fereño-Cáceres[A–F], Rómulo Vélez-Astudillo[A–F], Wilson Bravo-Torres[C–F], Daniela Astudillo-Rubio[C–F], Jacinto Alvarado-Cordero[E,F]

Postgraduate in Periodontology II Cohort, Faculty of Dentistry, University of Cuenca, Ecuador

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(4):605–612

**Address for correspondence**
Alejandro Fereño-Cáceres
E-mail: odfereno@gmail.com

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on February 1, 2023
Reviewed on April 4, 2023
Accepted on April 10, 2023

Published online on August 30, 2024

## Abstract

Currently, a new non-subtractive drilling technique, called osseodensification (OD), has been developed. It involves using specially designed drills with large negative cutting angles that rotate counterclockwise, causing expansion through plastic bone deformation, thus compacting the autologous bone to the osteotomy walls, which improves the primary stability of the implant.

The present systematic review aimed to determine whether the OD technique can increase the primary stability of dental implants in the posterior maxilla region as compared to the conventional drilling (CD) technique.

Five databases were searched up to June 30, 2022. The inclusion criteria embraced observational clinical studies, randomized and non-randomized controlled trials, human studies in vivo, comparing OD and CD, with the measurement of the primary stability of implants in the posterior maxilla region by means of the implant stability quotient (ISQ). The tools used to assess the risk of bias were RoB 2 and the Newcastle–Ottawa Scale (NOS).

Seven articles met the inclusion criteria, with 4 classified as having a low risk of bias and 3 with a moderate risk of bias. The OD technique consistently demonstrated an average ISQ value of 73 KHz across all studies, whereas CD yielded an average value of 58.49 kHz ($p < 0.001$ for 5 articles).

It can be concluded that in comparison with CD, OD improves primary stability at baseline in low-density bone, such as the maxilla.

**Keywords:** dental implant, primary stability, osseodensification, low-density bone

**Cite as**
Fereño-Cáceres A, Vélez-Astudillo R, Bravo-Torres W, Astudillo-Rubio D, Alvarado-Cordero J. Primary stability with osseodensification drilling of dental implants in the posterior maxilla region in humans: A systematic review. *Dent Med Probl.* 2024;61(4):605–612. doi:10.17219/dmp/163136

**DOI**
10.17219/dmp/163136

# Introduction

Dental implants have become the first choice for replacing missing teeth due to the predictable results of the procedure.[1] However, success depends mainly on the anchorage of dental implants in the surrounding bone, which is called osseointegration. The term was introduced by orthopedic surgeon and researcher Per-Ingvar Brånemark, who defined osseointegration as a direct and functional connection between the bone and the surface of a load-bearing implant at the microscopic level.[2–5]

For osseointegration to occur, it is essential to achieve primary stability through the initial mechanical coupling of the implant with the cortical bone. Obtaining osseointegration depends on the shape of the implant, the surgical technique and bone density.[5]

Various protocols have been proposed to assess primary implant stability, such as Periotest® measurements, insertion torque assessment, experimental implant removal, or resonance frequency analysis (RFA).[6]

Modern RFA devices, such as the Osstell device (W&H Dentalwerk Bürmoos, Bürmoos, Austria), provide an assessment of the primary stability of dental implants. These devices measure the implant resonance frequency in kilohertz (kHz), allowing the implant stability quotient (ISQ) to be calculated.[6]

The ISQ scale comprises values from 0 to 100. The values are not distributed linearly; values below 60 kHz correspond to low stability, values between 60 kHz and 69 kHz indicate medium stability, and values above 70 kHz correspond to high stability. Values greater than 68 are considered predictive of successful osseointegration.[6–8]

According to Lekholm and Zarb, the oral cavity contains different types of bone classified by bone quality: Class 1 – almost exclusively homogeneous compact bone; Class 2 – wide, compact bone surrounding dense cancellous bone; Class 3 – thin cortex surrounding dense cancellous bone; and Class 4 – thin cortex surrounding sparse cancellous bone.[9] Recent scientific research has shown implant failure rates for different bone types: 3.38% (Class 1); 3.13% (Class 2); 4.27% (Class 3); and 8.06% (Class 4), indicating that Class 4 bone has the highest failure rate.[10] This type of bone is frequently found in the maxilla in the posterior area, which is considered a structure of low bone density, where a reduced success rate in the placement of dental implants has been demonstrated.[11,12]

Generally, conventional drilling (CD) is used to prepare the implant bed. It involves using drills of different diameters and heights to cut and remove bone tissue.[13] Currently, a new non-subtractive drilling technique, called osseodensification (OD), has been developed. It involves using specially designed drills with large negative cutting angles that rotate counterclockwise, causing expansion through plastic bone deformation, thus compacting the autologous bone to the osteotomy walls, which improves the primary stability of the implant.[14,15]

The literature reports that OD brings promising and encouraging clinical results due to a significant increase in bone biomechanical properties as compared to CD, achieving high primary stability and a higher survival rate of dental implants in the short term; however, studies evaluating the success of OD have mostly been performed based on animal models and in vitro demonstrations.[16–18]

The purpose of the present study was to perform a systematic review addressing the following research question: Does the use of OD increase the primary stability of dental implants placed in the posterior maxilla region in humans as compared to CD?

# Methods

The methodology used in this research adheres to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) 2020 statement.[19]

## Eligibility, inclusion and exclusion criteria

The PICO framework was as follows: P – patients requiring implant placement; I – intervention (OD); C – comparison (CD); and O – outcome (ISQ).

The inclusion criteria comprised human studies in vivo, dental implants placed in the posterior maxilla region, studies comparing OD with CD, observational clinical studies (cases and controls), randomized and non-randomized controlled trials, and studies that measured the primary stability of dental implants with the use of ISQ.

The exclusion criteria comprised studies evaluating only OD, studies comparing OD with techniques other than CD, case reports, case reviews, review articles, systematic reviews, meta-analyses, case surveys, in vitro studies, studies conducted in animals or in synthetic sites (a dental typodont, or acrylic or plastic models), and studies that did not measure primary implant stability.

## Sources of information

The search for information was conducted up to June 30, 2022, in 5 databases – PubMed via MEDLINE, Google Scholar, Cochrane, Scopus, and ScienceDirect.

## Search strategy

An exhaustive bibliographic search was performed in the abovementioned databases to identify relevant articles published up to June 30, 2022, without limitations with regard to the language or the year of publication. The search strategies used keywords based on the PICO question, separated with the Boolean operator OR and combined with the Boolean operator AND. Additionally, gray literature was searched through the bibliographies of the articles included in this research.

The database search was carried out independently by two of the authors. The keywords used were: "dental implant"; "low density"; "implant"; "dental implants"; "dental implantation"; "endosseous implants"; "osseodensification"; "osseodensification osteotomy"; "versah drills"; "versah burs"; "osteotomy"; "implant osteotomy"; "drill"; "regular drilling"; "conventional drilling"; "peri-implant marginal bone loss"; "marginal bone loss"; "crestal bone loss"; "bone loss"; "bone remodeling"; and "primary stability" (Table 1).

## Study selection

The studies identified by the search were independently examined in duplicate by two investigators to assess their adherence to the inclusion criteria based on titles and abstracts. Articles on which both authors agreed, achieving a kappa concordance value of 0.937, were selected.

The full text of the selected articles was read to evaluate their titles and abstracts. Then, the references listed in all the selected articles were reviewed manually and compared with the inclusion criteria. Any disagreement regarding inclusion was resolved through discussion with a third author.

Scientific articles that met the inclusion criteria were selected and read in full text to evaluate the methodological quality of observational clinical studies and randomized and non-randomized controlled clinical trials comparing OD and CD in living humans. The assessment focused on the primary stability of dental implants placed in the maxilla, with low bone density, in terms of ISQ values.

## Data extraction

A data extraction protocol was defined; the form was elaborated in Excel (Office Excel 2011 software; Microsoft Corporation, Redmond, USA) and included authors, year, study design, number of participants, number of implants placed, and ISQ results for each technique used.

## Risk of bias assessment for individual studies

Version 2 of the Cochrane risk-of-bias tool (RoB 2) was used to assess the risk of bias in randomized clinical trials (RCTs).[20] The Newcastle–Ottawa Scale (NOS) was used to assess the risk of bias in non-randomized studies, including observational studies.[21] The 2 tools rated the included articles as having a low, moderate or high risk of bias.

**Table 1.** Electronic databases and search strategy

| Database | Search strategy |
|---|---|
| PubMed | P #1 ((dental implant) OR (low density) OR (implant) OR (dental implants) OR (dental implantation) OR (endosseous implants)) AND<br>I #2 ((osseodensification) OR (osseodensification osteotomy) OR (versah drills) OR (versah burs)) AND<br>C #3 ((osteotomy) OR (implant osteotomy) OR (drill) OR (regular drilling) OR (conventional drilling)) AND<br>O #4 ((peri implant marginal bone loss) OR (marginal bone loss) OR (crestal bone loss) OR (bone loss) OR (crestal bone loss) OR (bone remodeling) OR (primary stability))<br>#1 AND #2 AND #3 AND #4 |
| Google Scholar | P #1 "low density bone" OR "dental implant" AND<br>I #2 "osseodensification" OR "densah burs" OR "versah burs" AND<br>C #3 "conventional drilling" AND<br>O #4 "osseointegration" OR "peri-implant bone loss" OR "marginal bone loss" OR "radiographic evaluation" OR "primary stability"<br>#1 AND #2 AND #3 AND #4 |
| Cochrane | P #1 (dental implant) OR (low density) OR (endosseous implants) OR (dental implantation) OR (dental implants) in title abstract keyword AND<br>I #2 (osseodensification) OR (osseodensification osteotomy) OR (versah drills) OR (versah burs) OR (densah) in title abstract keyword AND<br>C #3 (osteotomy) OR (implant osteotomy) OR (drill) or (conventional drilling) OR (regular drilling) in title summary keyword AND<br>O #4 (peri implant marginal bone loss) OR (marginal bone loss) OR (crestal bone loss) OR (radiographic evaluation) OR (osseointegration) OR (primary stability)<br>#1 AND #2 AND #3 AND #4 |
| Scopus | P #1 (title-abs-key (dental and implant) OR title-abs-key (low and density) OR title-abs-key (dental and implants) OR title-abs-key (dental and implantation) OR title-abs-key (endosseous and implants)) AND<br>I #2 (title-abs-key (osseodensification) OR title-abs-key (osseodensification and osteotomy) OR title-abs-key (versah and drills) OR title-abs-key (versah and burs ) OR title-abs-key (densah)) AND<br>C #3 (title-abs-key (osteotomy) OR title-abs-key (implant and osteotomy) OR title-abs-key (drill) OR title-abs-key (regular and drilling) OR title-abs-key (conventional and drilling)) AND<br>O #4 (title-abs-key (periimplant and marginal and bone and loss) OR title-abs-key (crestal and bone and loss) OR title-abs-key (bone and remodeling) OR title-abs-key (osseointegration) OR title-abs-key (radiographic and evaluation) OR title-abs-key (primary and stability))<br>#1 AND #2 AND #3 AND #4 |
| ScienceDirect | P #1 ("low density bone" OR "dental implant") AND<br>I #2 ("osseodensification" OR "versah burs") AND<br>C #3 ("conventional drilling") AND<br>O #4 ("osseointegration" OR "marginal bone loss" OR "radiographic evaluation" OR "primary stability") |
| Manual search | dental implant, low density, dental implantation, endosseous implants, osseodensification, osseodensification osteotomy, versah drills, versah burs, implant osteotomy, regular drilling, conventional drilling, primary stability |

PICO strategy: P – population; I – intervention; C – comparison; and O – outcome.

# Results

## Search and selection

The systematic search yielded a total of 196 articles; after filtering out duplicate articles and articles without access, 129 were obtained; 30 were discarded based on the title and abstract. According to the inclusion and exclusion criteria, 93 articles were eliminated. Through the manual search of the bibliography of each article and relevant reviews, 1 article was included, resulting in a total of 7 articles included in the present review.

The PRISMA 2020 flowchart summarizing the selection process is shown in Fig. 1.

## Comparison of the drilling techniques

In the included articles, the OD technique presented ISQ values greater than 68 kHz, which is the minimum value to achieve successful osseointegration, as compared to the CD technique with an average ISQ value of 58.49 kHz.[7,11,15,17,22–24] Five articles showed $p < 0.001$ in this respect[7,15,17,22,24] and in 2 articles, the $p$-value was >0.001.[11,23] In addition, it must be taken into account that OD is a technique that uses almost the same protocol with regard to the drilling speed as CD, the difference being that the direction is counterclockwise. All this shows predictable parameters for successful osseointegration in comparison with CD. It should also be mentioned that in most of the included studies, the patients were between 50 and 60 years of age (Table 2).

## Risk of bias for individual studies

Among the 7 studies included in the systematic review, 4 were assessed to have a low risk of bias, while 3 had a moderate risk of bias. None of the articles was found to have a high risk of bias. The moderate risk of bias was attributed to the randomization process and the selection of the reported outcomes (Tables 3 and 4).

# Discussion

The primary or initial stability of dental implants results from the mechanical union with the peri-implant bone, and is considered a primordial factor in achieving secondary stability with subsequent osseointegration.[23] It is a priority to employ an adequate surgical technique to improve the primary stability of the implant and to adapt to the existing protocols to achieve successful osseointegration, even more so in cases where bone quality is not optimal.[25,26]

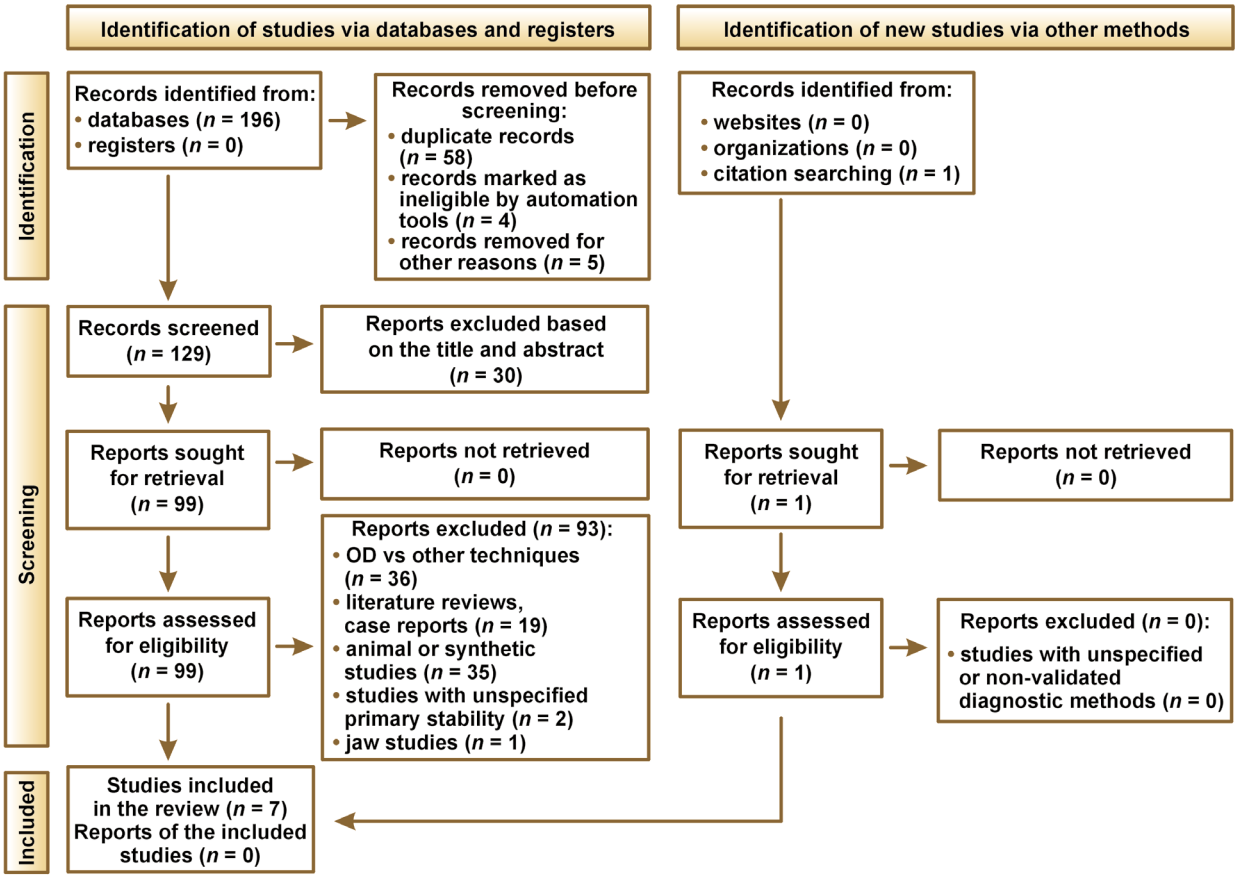


Fig. 1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) 2020 flowchart

OD – osseodensification.

**Table 2.** Summary of the studies included in the systematic review

| Study | Study design | Number of patients | Patient demographics | Number of implants | Manufacturer | Implant surface or type | Osteotomy speed [rpm] | Implant insertion speed [rpm] | Implant IT [N•m] | ISQ *M* ±*SD* | *p*-value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bergamo et al. 2021[7] | multi-center controlled clinical trial, split-mouth, double-blind | 56 | 26 M/30 F mean age: 54.2 ±3.5 years | 150 | Strong SW Plus (S.I.N. Implant System, São Paulo, Brazil; Zimmer Biomet, Warsaw, USA) and IS-III Active (NeoBiotech USA, Pasadena, USA) | an internal conical connection, tapered macrogeometry and a sandblasted acid-etched surface | CD: 1,100 OD: 1,100 (with saline irrigation) | CD: 20–50 OD: 20–50 (without irrigation) | CD: 35 OD: 54 | CD: 62 ±2.0 OD: 73 ±2.0 | <0.001* |
| Al Ahmari 2022[11] | multi-center clinical study | 20 | age: 40–59 years | CD: 20 OD: 20 | BioHorizons Implant Systems, Inc., Birmingham, USA | NR | NR | NR | NR | CD: 50.00 ±11.23 OD: 50.57 ±11.23 | >0.001 |
| Mello-Machado et al. 2021[15] | RCT | 30 | 7 M/23 F mean age: 50.0 ±9.3 years | CD: 26 OD: 29 | Emfils, São Paulo, Brazil | Morse™ platform | CD: 1,200 OD: 1,200 (with saline irrigation) | NR | CD: 32.0 ±3.4 OD: 39.0 ±6.4 | CD: 65.5 ±2.7 OD: 67.1 ±3.2 | <0.001* |
| Ibrahim et al. 2020[17] | clinical trial | 10 | 5 M/5 F mean age: 36.0 ±4.1 years | 20 | Dentium Co., Ltd, Suwon, South Korea | a conical hex implant–abutment connection, a round tapered design and implant healing abutments | CD: 800–1,200 OD: 800–1,500 (with saline irrigation) | NR | NR | CD: 59.65 ±5.39 OD: 74.25 ±4.95 | <0.001* |
| Abdelwahab et al. 2022[22] | observational study | 10 | 10 M/0 F age: 50–65 years | CD: 10 OD: 10 | NR | NR | NR | NR | NR | CD: 60.67 ±8.95 OD: 67.67 ±5.87 | <0.001* |
| Sultana et al. 2020[23] | observational study | 20 | NR | CD: 10 OD: 10 | Touareg™-S (Adin Dental Implant Systems, Ltd., Afula, Israel) | spiral dental implants | CD: 800–1,000 OD: 800–1,500 (with copious irrigation | NR | NR | CD: 57.6 OD: 65.6 | >0.05 |
| Kothayer and Abdelfattaha 2020[24] | observational study | 10 | 10 M/0 F age: 45–60 years | CD: 10 OD: 10 | NucleOSS, Izmir, Turkey | NR | CD: 800 OD: 800 (with copious irrigation) | NR | NR | CD: 55.75 ±10.44 OD: 67.63 ±5.73 | <0.001* |

*M* – mean; *SD* – standard deviation; IT – insertion torque; ISQ – implant stability quotient; RCT – randomized clinical trial; M – male; F – female; CD – conventional drilling; NR – not reported; * statistically significant.

**Table 3.** Risk of bias of the included studies according to the Newcastle–Ottawa Scale (NOS)

| Study | Selection | Compatibility | Outcomes | Score |
|---|---|---|---|---|
| Abdelwahab et al. 2022[22] | *** | * | *** | low risk |
| Sultana et al. 2020[23] | *** | * | *** | low risk |
| Kothayer and Abdelfattaha 2020[24] | **** | * | *** | low risk |

Therefore, the purpose of this study was to conduct a systematic review addressing the research question: Does the use of OD increase the primary stability of dental implants placed in the posterior maxilla region in humans as compared to CD?

An alternative to CD has been proposed, since this conventional technique eliminates bone particles in the surgical act, whereas OD keeps bone particles compact in the osteotomy wall.[14] This offers mechanical and biological advantages in terms of intimate contact between the implant and the bone because of residual bone fragments on the implant surface and improved bone healing due to the nucleation of osteoblasts in the instrumented bone.[27–29]

Furthermore, a major advantage of the OD approach is the ability, in numerous scenarios, to circumvent the need for bone and membrane grafts. This not only reduces surgical invasiveness and significantly shortens the healing time, but also helps in minimizing peri-implant inflammation, which is often greater in implants placed in the native bone. From this point of view, it is necessary to underline the importance of keeping the level of peri-implant bone inflammation as limited as possible, as emphasized by Guarnieri et al.[30,31]

Numerous surgical procedures have been suggested to avoid or decrease bone loss during implant placement in low-density bone, and to improve the primary stability of the implant and bone quality. However, few investigators recommended decreasing the osteotomy site with regard to the implant diameter by approx. 10% to decrease bone stress and improve primary implant stability. The osteotomy site decreased by more than 10% does not provide any mechanical benefit. However, the OD approach increases the primary stability of the implant.[7–10]

Human studies can be extrapolated to daily clinical practice; therefore, it should be clarified that this review is the first to focus on primary stability in low-density bone. Several authors have demonstrated favorable and predictable results with OD as compared to CD, i.a., Abdelwahab et al.[22] and Kothayer et al.,[24] who mentioned superior primary stability with statistically significant values ($p < 0.001$).

Furthermore, the advantages of OD over CD were mentioned by Mello-Machado et al., who did not need to increase the drilling diameter for osteotomy, allowing the placement of a 3.8-millimeter implant in an ideal space for an implant with a 3.5-millimeter diameter.[15] Similarly, Al Ahmari evidenced an increase in peri-implant bone density when using OD as compared to CD.[11] These results are associated with the elastic compression of the bone tissue toward the implant due to the recovery effect of the implant as a consequence of OD drilling. In this way, the implant compresses the bone and, at the same time, the bone compresses the implant inversely.[14]

The dimensions of implants vary in each technique. Ibrahim et al. showed the superiority of OD in terms of primary stability as compared to CD with $p < 0.001$, but the implants used for CD were 3.8 mm and for OD 5.0 mm in diameter.[17] Conversely, Bergamo et al. evaluated the primary stability of 150 implants with a statistically superior ISQ in OD at $p < 0.001$; however, they did not achieve the same results with short implants.[7]

The results of the present systematic review demonstrated an average ISQ value of 73 kHz for the OD technique, which is higher than the minimum value to achieve successful osseointegration (68 kHz). The CD technique obtained an average ISQ value of 58.49 kHz. Therefore, OD, due to its non-subtractive nature, may be useful in sites with a larger trabecular bone volume, such as the

**Table 4.** Risk of bias of the included studies according to RoB 2

| Study | Study ID | Experimental | Comparator | Outcome | Weight | D1 | D2 | D3 | D4 | D5 | Overall |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Bergamo et al. 2021[7] | NA | NA | NA | implant stability | 1 | + | + | + | + | + | + |
| Al Ahmari 2022[11] | NA | NA | NA | implant stability | 1 | ! | + | + | + | ! | ! |
| Mello-Machado et al. 2021[15] | NA | NA | NA | implant stability | 1 | ! | + | + | + | + | ! |
| Ibrahim et al. 2020[17] | NA | NA | NA | implant stability | 1 | + | + | + | + | ! | ! |

D1 – randomization process; D2 – deviations from the intended interventions; D3 – missing outcome data; D4 – measurement of the outcome; D5 – selection of the reported outcomes; NA – not available.
Risk of bias: + low; ! moderate (some concerns); – high.

maxilla, thus improving bone mineral density and potentially accelerating osseointegration due to elastic recovery.[32] Yet, 2 articles in this review did not show immediate statistical significance for dental implant placement with OD, i.e., by Al Ahmari, with a mean ISQ value of 50.57 ±11.23 ($p > 0.001$),[11] and by Sultana et al., with an ISQ value of 65.6 ($p > 0.05$).[23]

Future prospective research on the use of the OD technique for immediate loading is recommended to achieve faster rehabilitation of stomatognathic system functions without compromising dental implant success, as mentioned by Krawiec et al.[33] Similarly, changes at the peri-implant bone level may occur during healing, as noted by Abarno et al.,[34] where dental implants bear tension and stress upon prosthetic loading. Therefore, the results obtained with regard to primary stability can lead to studies on the predictability of OD in relation to the abovementioned events.

## Limitations

As a limitation of the present study, it should be noted that there were few studies involving human subjects, primarily due to bioethical concerns and the fact that OD is a relatively new technique developed in the last decade. Therefore, only limited data could be obtained regarding the primary stability of implants placed in the posterior maxilla region.

# Conclusions

The obtained data showed significantly higher ISQ values for primary stability in the posterior maxilla region, favoring OD over CD. However, these results should be interpreted cautiously due to the limited evidence available on the use of this technique in humans.

## Registration

The study protocol was registered at https://inplasy.com under the number 202290066.

## Ethics approval and consent to participate

Not applicable.

## Data availability

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

## Consent for publication

Not applicable.


## ORCID iDs

Alejandro Fereño-Cáceres https://orcid.org/0000-0002-1067-9606
Rómulo Vélez-Astudillo https://orcid.org/0000-0003-0788-0293
Wilson Bravo-Torres https://orcid.org/0000-0002-9431-1808
Daniela Astudillo-Rubio https://orcid.org/0000-0002-8154-0492
Jacinto Alvarado-Cordero https://orcid.org/0000-0002-5615-5314


## References


1. Sakka S, Baroudi K, Nassani MZ. Factors associated with early and late failure of dental implants. *J Investig Clin Dent*. 2012;3(4):258–261. doi:10.1111/j.2041-1626.2012.00162.x
2. Brånemark R, Brånemark PI, Rydevik B, Myers RR. Osseointegration in skeletal reconstruction and rehabilitation: A review. *J Rehabil Res Dev*. 2001;38(2):175–181. PMID:11392650.
3. Giudice A, Bennardo F, Antonelli A, et al. Influence of clinician's skill on primary implant stability with conventional and piezoelectric preparation techniques: An ex-vivo study. *J Biol Regul Homeost Agents*. 2020;34(2):739–745. doi:10.23812/20-96-L-53
4. Albrektsson T, Albrektsson B. Osseointegration of bone implants. A review of an alternative mode of fixation. *Acta Orthop Scand*. 1987;58(5):567–577. doi:10.3109/17453678709146401
5. Davies JE. Understanding peri-implant endosseous healing. *J Dent Educ*. 2003;67(8):932–949. doi:10.1002/j.0022-0337.2003.67.8.tb03681.x
6. Kästel I, De Quincey G, Neugebauer J, Sader R, Gehrke P. Does the manual insertion torque of smartpegs affect the outcome of implant stability quotients (ISQ) during resonance frequency analysis (RFA)? *Int J Implant Dent*. 2019;5(1):42. doi:10.1186/s40729-019-0195-1
7. Bergamo ET, Zahoui A, Barrera RB, et al. Osseodensification effect on implants primary and secondary stability: Multicenter controlled clinical trial. *Clin Implant Dent Relat Res*. 2021;23(3):317–328. doi:10.1111/cid.13007
8. Farzad P, Andersson L, Gunnarsson S, Sharma P. Implant stability, tissue conditions, and patient self-evaluation after treatment with osseointegrated implants in the posterior mandible. *Clin Implant Dent Relat Res*. 2004;6(1):24–32. doi:10.1111/j.1708-8208.2004.tb00024.x
9. Lekholm U, Zarb G. Patient selection and preparation. In: Bränemark PI, Zarb GA, Albreksson T (ed.). *Tissue-Integrated Prostheses: Osseointegration in Clinical Dentistry*. Chicago, IL: Quintessence Publishing Co., Inc.; 1985:199–209.
10. Chrcanovic B, Albrektsson T, Wennerberg A. Bone quality and quantity and dental implant failure: A systematic review and meta-analysis. *Int J Prosthodont*. 2017;30(3):219–237. doi:10.11607/ijp.5142
11. Al Ahmari NM. Osseo-densification versus conventional surgical technique in low density jaw bone: A split mouth in vivo study. *Technol Health Care*. 2022;30(5):1117–1124. doi:10.3233/THC-220048
12. Martinez H, Davarpanah M, Missika P, Celletti R, Lazzara R. Optimal implant stabilization in low density bone. *Clin Oral Implants Res*. 2001;12(5):423–432. doi:10.1034/j.1600-0501.2001.120501.x
13. Witek L, Neiva R, Alifarag A, et al. Absence of healing impairment in osteotomies prepared via osseodensification drilling. *Int J Periodontics Restorative Dent*. 2019;39(1):65–71. doi:10.11607/prd.3504
14. Huwais S, Meyer EG. A novel osseous densification approach in implant osteotomy preparation to increase biomechanical primary stability, bone mineral density, and bone-to-implant contact. *Int J Oral Maxillofac Implants*. 2017;32(1):27–36. doi:10.11607/jomi.4817
15. Mello-Machado RC, De Almeida Barros Mourão CF, Javid K, Ferreira HT, Montemezzi P, Calasans-Maia MD, Senna PM. Clinical assessment of dental implants placed in low-quality bone sites prepared for the healing chamber with osseodensification concept: A double-blind, randomized clinical trial. *Appl Sci*. 2021;11(2):640. doi:10.3390/app11020640
16. Tretto PH, Fabris V, Cericato GO, Sarkis-Onofre R, Bacchi A. Does the instrument used for the implant site preparation influence the bone–implant interface? A systematic review of clinical and animal studies. *Int J Oral Maxillofac Surg*. 2019;48(1):97–107. doi:10.1016/j.ijom.2018.04.005
17. Ibrahim AM, Ayad SS, ElAshwah A. The effect of osseodensification technique on implant stability (clinical trial). *Alex Dent J*. 2020;45(2):1–7. doi:10.21608/ADJALEXU.2020.86758

18. Gaikwad AM, Joshi AA, Nadgere JB. Biomechanical and histomorphometric analysis of endosteal implants placed by using the osseodensification technique in animal models: A systematic review and meta-analysis. *J Prosthet Dent*. 2022;127(1):61–70. doi:10.1016/j.prosdent.2020.07.004
19. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *Syst Rev*. 2021;10(1):89. doi:10.1186/s13643-021-01626-4
20. Sterne JA, Savović J, Page MJ, et al. RoB 2: A revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019;366:l4898. doi:10.1136/bmj.l4898
21. Wells GA, Shea B, O'Connell D, et al. The Newcastle–Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed October 13, 2022.
22. Abdelwahab K, Nawwar Alaa, El Khourazaty N. Assessment of primary stability and bone density of maxillary implant overdenture following osseodensification concept. *Egypt Dent J*. 2022;68(1):955–963. doi:10.21608/EDJ.2022.109760.1896
23. Sultana A, Makkar S, Saxena D, Wadhawan A, Kusum CK. To compare the stability and crestal bone loss of implants placed using osseodensification and traditional drilling protocol: A clinicoradiographical study. *J Indian Prosthodont Soc*. 2020;20(1):45–51. doi:10.4103/jips.jips_133_19
24. Kothayer M, Abdelfattaha A. Effect of using Densah burs on implant stability and peri-implant marginal bone loss in maxillary implant supported partial overdentures. *Egypt J Oral Maxillofac Surg*. 2020;11(1):27–35. doi:10.21608/omx.2020.28640.1062
25. Bonfante EA, Jimbo R, Witek L, et al. Biomaterial and biomechanical considerations to prevent risks in implant therapy. *Periodontol 2000*. 2019;81(1):139–151. doi:10.1111/prd.12288
26. Stocchero M, Toia M, Cecchinato D, Becktor JP, Coelho PG, Jimbo R. Biomechanical, biologic, and clinical outcomes of undersized implant surgical preparation: A systematic review. *Int J Oral Maxillofac Implants*. 2016;31(6):1247–1263. doi:10.11607/jomi.5340
27. Lahens B, Neiva R, Tovar N, et al. Biomechanical and histologic basis of osseodensification drilling for endosteal implant placement in low density bone. An experimental study in sheep. *J Mech Behav Biomed Mater*. 2016;63:56–65. doi:10.1016/j.jmbbm.2016.06.007
28. Jimbo R, Tovar N, Marin C, et al. The impact of a modified cutting flute implant design on osseointegration. *Int J Oral Maxillofac Surg*. 2014;43(7):883–888. doi:10.1016/j.ijom.2014.01.016
29. Lopez CD, Alifarag AM, Torroni A, et al. Osseodensification for enhancement of spinal surgical hardware fixation. *J Mech Behav Biomed Mater*. 2017;69:275–281. doi:10.1016/j.jmbbm.2017.01.020
30. Guarnieri R, Reda R, Di Nardo D, Miccoli G, Zanza A, Testarelli L. Clinical, radiographic, and biochemical evaluation of two-piece versus one-piece single implants with a laser-microgrooved collar surface after 5 years of functional loading. *Clin Implant Dent Relat Res*. 2022;24(5):676–682. doi:10.1111/cid.13118
31. 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
32. Padhye NM, Padhye AM, Bhatavadekar NB. Osseodensification – a systematic review and qualitative analysis of published literature. *J Oral Biol Craniofac Res*. 2020;10(1):375–380. doi:10.1016/j.jobcr.2019.10.002
33. Krawiec M, Olchowy C, Kubasiewicz-Ross P, Hadzik J, Dominiak M. Role of implant loading time in the prevention of marginal bone loss after implant-supported restorations: A targeted review. *Dent Med Probl*. 2022;59(3):475–481. doi:10.17219/dmp/150111
34. Abarno S, Gehrke AF, Dedavid BA, Gehrke SA. Stress distribution around dental implants, generated by six different ceramic materials for unitary restoration: An experimental photoelastic study. *Dent Med Probl*. 2021;58(4):453–461. doi:10.17219/dmp/135997

Review

# Movement disorders of the stomatognathic system: A blind spot between dentistry and medicine

Kazuya Yoshida[A–F]

Department of Oral and Maxillofacial Surgery, National Hospital Organization, Kyoto Medical Center, Kyoto, Japan

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*. 2024;61(4):613–625

**Address for correspondence**
Kazuya Yoshida
E-mail: yoshida.kazuya.ut@mail.hosp.go.jp;
omdystonia@gmail.com

**Funding sources**
The study was supported by grants from the Japanese Ministry of Health, Labor and Welfare (19K102370001).

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on January 27, 2024
Reviewed on February 5, 2024
Accepted on February 22, 2024

Published online on August 30, 2024

## Abstract

Movement disorders of the stomatognathic system include oromandibular dystonia (OMD), oral dyskinesia, sleep/awake bruxism, functional (psychogenic) stomatognathic movement disorders (FSMDs), tremors, and hemimasticatory spasm (HMS). Most patients first consult dentists or oral surgeons. The differential diagnoses of these involuntary movements require both neurological and dental knowledge and experience, and some of these movement disorders are likely to be diagnosed as bruxism or temporomandibular disorders (TMDs) by dental professionals. However, excepting movement disorder specialists, neurologists may find it difficult to differentially diagnose these disorders. Patients may visit numerous medical and dental specialties for several years until a diagnosis is made. Therefore, movement disorders of the oral region may represent a blind spot between dentistry and medicine.

The present narrative review aimed to describe the clinical characteristics and differential diagnoses of some movement disorders, as well as the problems bridging dentistry and medicine. Movement disorders have the following characteristic clinical features: OMD – task specificity, sensory tricks and the morning benefit; FSMDs – inconsistent and incongruous symptoms, spreading to multiple sites and the lack of sensory tricks; and HMS – the paroxysmal contraction of unilateral jaw closing muscles, the persistence of symptoms during sleep and the loss of a silent period. A careful differential diagnosis is essential for the adequate and effective treatment of each involuntary movement. Refining the latest definition of bruxism may be necessary to prevent the misdiagnosis of involuntary movements as bruxism.

Both dental and medical professionals should take an interest in the movement disorders of the stomatognathic system, and these disorders should be diagnosed and treated by a multidisciplinary team.

**Keywords:** differential diagnosis, bruxism, movement disorder, oromandibular dystonia, hemimasticatory spasm

**Cite as**
Yoshida K. Movement disorders in the stomatognathic system: A blind spot between dentistry and medicine. *Dent Med Probl.* 2024;61(4):613–625. doi:10.17219/dmp/185249

**DOI**
10.17219/dmp/185249

## Introduction

The stomatognathic system is an anatomical and functional unit comprising hard tissues (bones constructing the mandible and maxilla, teeth, and temporomandibular joints) and soft tissues (masticatory, tongue, lip, cheek, and lower facial muscles, as well as nervous and vascular supplies). This system plays an indispensable role in various important functions, such as mastication, swallowing, speech, breathing, and facial expressions. Dentists and oral surgeons who specialize in the stomatognathic system were the first healthcare professionals to observe patients exhibiting symptoms in this region. Various movement disorders exist within the stomatognathic system, including oromandibular dystonia (OMD), orolingual dyskinesia, bruxism, functional (psychogenic) stomatognathic movement disorders (FSMDs), hemimasticatory spasm (HMS), tremors, tics, and myokymia.[1] Moreover, several other diseases exhibit symptoms in the stomatognathic system, including Parkinson's disease, Down syndrome, Tourette syndrome, Rett syndrome, autistic spectrum disorders, Huntington's disease, Wilson's disease, chorea-acanthocytosis, Leigh syndrome, and Lesch–Nyhan syndrome.[2–4] In one study, approx. 70% of patients with OMD saw dentists, and 60% visited oral surgeons.[5] However, around 90% of these patients were not properly diagnosed[5]; instead, they were diagnosed with bruxism, temporomandibular disorders (TMDs) or psychiatric diseases.[1,5–8] According to the current definition of bruxism,[9] some patients with movement disorders may be misdiagnosed.[1,7] Patients continue to receive ineffective treatment for misdiagnosed bruxism or TMDs, which sometimes leads to the discontinuation of further treatment.[1,7,8] Medical professionals may misdiagnose bruxism or TMDs as other involuntary movements, such as jaw closing dystonia or jaw deviation dystonia.[1,7] Thus, these professionals cannot always adequately diagnose and treat the movement disorders of the stomatognathic system.[1,7,8]

The diagnosis and treatment of involuntary movements in the stomatognathic region require both medical and dental knowledge and experience. Dental professionals often lack knowledge about involuntary movements, such as dystonia, whereas medical professionals typically lack familiarity with the anatomy and function of the stomatognathic region. Therefore, this condition represents a blind spot in both dentistry and medicine. Furthermore, many attending physicians are unaware of their inability to properly diagnose and treat involuntary movements in the stomatognathic region.

The purpose of the present review was to outline involuntary movements in the stomatognathic region, particularly movement disorders that need to be differentiated from bruxism, such as OMD, FSMDs and HMS, explain their clinical signs and differential diagnoses, and discuss related problems in dentistry and medicine.

## Material and methods

The literature search embraced electronic medical literature databases (PubMed, Embase, the Web of Science, Google Scholar, the Japan Medical Abstracts Society, and Medical Online) and used the following keywords: "movement disorder"; "sleep bruxism"; "awake bruxism"; "temporomandibular disorder"; "oromandibular dystonia"; "jaw closing dystonia"; "oral dyskinesia"; "differential diagnosis"; "hemimasticatory spasm"; "functional movement disorder"; "etiology"; "epidemiology"; and "treatment". Additionally, a manual search was conducted to evaluate the articles cited in the related resources. Reports prior to November 30, 2023, identified in the abovementioned databases or via a manual search, with no language restrictions, were screened by the author.[9,10] The exclusion criteria were records irrelevant to the purpose of this study. All studies were evaluated and assessed for eligibility, and reviewed by the author.

## Clinical characteristics of involuntary movements in the stomatognathic system

### Bruxism

Bruxism is classified as either sleep or awake bruxism, which are defined as masticatory muscle activities that occur during sleep and wakefulness, respectively.[11] Sleep bruxism is further characterized as rhythmic or non-rhythmic, and awake bruxism is characterized by repetitive or sustained tooth contact and/or by bracing or thrusting of the mandible.[11] Sleep and awake bruxism are considered different behaviors during sleep and wakefulness.[11] Bruxism is an oral motor behavior that primarily includes teeth grinding and/or clenching. Sleep bruxism, primarily characterized by grinding, and awake bruxism, mainly manifesting as clenching, typically do not occur in the same individuals.[12] Bruxism can be distinguished as primary/idiopathic, secondary, and iatrogenic bruxism.[13] Primary/idiopathic bruxism is characterized by the absence of any identifiable medical or dental causes. However, secondary bruxism is associated with a medical/psychiatric condition such as a movement disorder, sleep-related disorder, or neurological or psychiatric disorder.[13] Iatrogenic bruxism follows drug intake or withdrawal including chemical substances and medications.[13]

#### Presentation

Bruxism has been associated with tooth wear or cracked teeth, failure of dental restorations, prostheses, or implants, pain in the masticatory muscles, teeth,

temporomandibular joints, TMDs, masseter muscle hypertrophy, tooth fracture, cheek or lip biting, hypersensitive teeth, and tension headaches. Indentations on the tongue, lips, and/or linea alba on the inner cheek are occasionally observed.

In otherwise healthy individuals, bruxism should not be considered a disorder but rather a behavior that can be a risk (and/or protective) factor for certain clinical consequences.[11] Additionally, a grading system for bruxism was proposed to determine the likelihood that a certain assessment of bruxism actually yields a valid outcome: 1) possible sleep/awake bruxism (based only on a positive self-report); 2) probable sleep/awake bruxism (based on a positive clinical inspection, with or without a positive self-report); and 3) definite sleep/awake bruxism (based on a positive instrumental assessment, with or without a positive self-report and/or positive clinical inspection).[11]

Although bruxism is generally assessed using self-reports or clinical findings, instrumental assessments are more reliable for diagnosing definite bruxism. Polysomnography is used as an instrumental assessment of sleep bruxism and is considered the gold standard for diagnosis.[14] Polysomnography with audio-video recording or ambulatory electromyography (EMG) devices has been applied for a definite diagnosis; future studies using ambulatory EMG instruments may shift focus to scoring the entire spectrum of masticatory muscle activity.[15] There is no definitive examination method for awake bruxism, but recently, ecological momentary assessment (EMA) has been applied. The EMA with a smartphone-based strategy has allowed data collection on the frequency of different awake bruxism behaviors reported by individuals in their natural environment.[16–20] Applications using EMA include BruxApp®,[16–20] WhatsApp with a web-based survey program called Mentimeter®,[21] and a time logger.[22] Early data were obtained from university students,[16] but subsequent studies have included data from the general population[17] and patients with bruxism.[22] A recent study applied simultaneous recording of EMA and EMG to patients with bruxism to obtain more definitive data on awake bruxism.[22] In the study, the association between probable and definite bruxism was confirmed. In the bruxism group, 30 out of 64 participants were definitively diagnosed and assigned to the control group. Additionally, 11 out of 36 participants were classified into the probable control group diagnosed with definite bruxism.[22]

## Epidemiology

Bruxism is a common condition, observed to some degree during the lifetime of approx. 85–90% of the general population; however, only 5% develop a clinical condition.[23] In earlier studies, the estimated prevalence of sleep bruxism awareness was based on reports by parents or sleep partners. Reports on the prevalence of awake bruxism vary widely, depending on the diagnostic method (possible or probable bruxism) and the patients investigated. According to an umbrella review, the prevalence of awake bruxism was 22–30%, and that of sleep bruxism was 1–15%.[24] Sex differences with respect to sleep bruxism are not obvious, and most studies report an equal prevalence in men and women.[25] However, awake bruxism occurs more frequently in women than in men.[26] Because other movement disorders occur more frequently (60–70%) in women, a higher incidence of awake bruxism in women suggests that other movement disorders may be diagnosed as awake bruxism. However, numerous reports on the epidemiology of bruxism are based on patient self-reports or clinical symptoms, and their reliability should be carefully evaluated.

## Etiology

Although the etiology of bruxism is not fully understood, a multifactorial etiology has been postulated, including biological, psychological (anxiety and stress), and exogenous (drugs, caffeine, tobacco, and alcohol) factors.[24,27] Sleep bruxism is characterized as a repetitive sleep-related movement disorder mainly involving rhythmic masticatory muscle activity at a frequency of 1 Hz, with occasional tooth grinding.[28–30] Most sleep bruxism episodes coincide with brief cardiac and brain reactivations known as micro-arousals. Rhythmic masticatory muscle activity results from a sequence of events associated with sleep micro-arousals.[28–30]

Bruxism can occur in conditions beyond neurological disorders. A review examining bruxism in movement disorders found the highest incidence in Rett syndrome (97%), Down syndrome (42%), and autism spectrum disorder (32%).[3]

## Treatment

Bruxism should not be recognized as a disorder in otherwise healthy individuals but rather as a behavior that can be a risk (and/or protective) factor for certain clinical consequences.[11] In most cases, active treatment is not necessary. Treatments for bruxism include medication, occlusal splints, physical therapy, biofeedback therapy, and cognitive behavioral therapy.[24] Recently, botulinum toxin therapy has been clinically applied.[1,31,32] Botulinum toxin therapy should be considered in severe cases when other traditional treatments are ineffective. Palpation, EMG, and occlusal force measurements are used to identify target muscles.[1,8,33,34] In most cases, botulinum toxin is injected into the masseter and temporalis muscles. If the effect diminishes and the medial pterygoid muscle is tender, botulinum toxin should be administered into the medial pterygoid muscle.[1,8,33,34] In cases with severe grinding, the lateral pterygoid muscle is often painful and may also be treated.[1,8,33,34] Prolonged, intense bruxism can lead to the development of excessive tendonous tissue at the anterior margin of the masseter muscle, resulting in

masticatory muscle tendon-aponeurosis hyperplasia.[35–37] In such cases, a coronoidotomy is necessary to improve limited mouth opening and muscle pain.[35,36]

## Oromandibular dystonia (OMD)

Dystonia is a hyperkinetic movement disorder characterized by sustained or intermittent muscle contractions that cause abnormal repetitive movements and/or postures.[2] Dystonia is categorized as focal, multifocal, segmental, hemidystonia, and generalized.[2] Oromandibular dystonia is a focal type of dystonia characterized by sustained, intermittent, or task-specific contractions of the masticatory, lingual, pharyngeal, and/or muscles of the stomatognathic system.[1,2,4–8,38]

### Presentation

Symptoms of OMD include masticatory disturbance, tongue biting, cheek biting, limited mouth opening, muscle pain, dysphagia, dysarthria, upper airway obstructions,[39] and temporomandibular joint dislocations.[39,40] These symptoms can significantly impair daily activities, cause social embarrassment and cosmetic disfigurement, and limit patients' ability to work, thereby having a profound impact on quality of life.[1,6] Oromandibular dystonia is classified into 6 subtypes: jaw closing; jaw opening; lingual; jaw deviation; jaw protrusion; and lip dystonia (Fig. 1).[1,6–8]

Oromandibular dystonia is generally diagnosed based on characteristic clinical features of focal dystonia and EMG findings.[1,6–8] These features include stereotypy, task specificity, sensory tricks, co-contraction, morning benefit, and overflow phenomenon.[1,6–8,38,41] Patients with OMD exhibit distinct stereotypical patterns of muscle contraction depending on the subtype. For instance, stereotypy was observed in 95.8% of 385 patients with OMD in one study.[7] During the initial phase, OMD symptoms often manifest only during specific tasks such as speaking, chewing, or mouth opening.[38] Notably, 69.9% of patients with OMD demonstrated task specificity.[7] Sensory tricks are sensory-based methods that can temporarily alleviate dystonia symptoms, such as chewing gum or candy.[7,41] Sensory tricks were observed in 51.4% of patients with OMD.[7] Symptoms of dystonia tend to be milder in the morning. This is called morning benefit and was reported in 47.3% of patients with OMD.[7] Co-contraction involves involuntary simultaneous contractions of agonist and antagonist muscles due to loss of reciprocal inhibition of muscular activities.[1,6–8] Dystonic contracture of masticatory muscles may extend to other muscles including the orbicularis oris, orbicularis oculi, or other facial, neck, and shoulder muscles, which is known as the overflow phenomenon.[38]

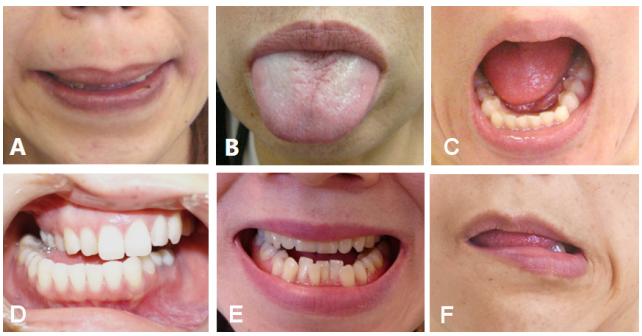


Fig. 1. Subtypes of oromandibular dystonia (OMD)

A – jaw closing dystonia; B – tongue dystonia; C – jaw opening dystonia; D – jaw deviation dystonia; E – jaw protrusion dystonia; F – lip dystonia.

### Epidemiology

The onset of OMD typically occurs in patients in their 50s.[1,42–44] However, drug-induced tardive OMD can occur in teenagers. Women are affected approx. twice as frequently as men.[42–44] Oromandibular dystonia can occur in isolation; however, it may present with other comorbidities, such as segmental or generalized dystonia. The ratio of isolated OMD among all OMD cases differs considerably, as reported by neurologists (focal – 39%; segmental – 43%; and generalized – 10%)[43] and oral surgeons (focal – 90.8%; segmental – 10.4%; and multifocal – 6.3%).[43] This difference may be attributed to neurologists primarily assessing OMD cases associated with neurological diseases, whereas oral surgeons were able to identify numerous mild cases.[44]

The prevalence of OMD has been shown to be considerably higher than previously estimated.[6,43] A recent study reported the prevalence of OMD to be 9.8 per 100,000 persons, with an incidence of 2 per 100,000 person-years.[43] The study suggested that OMD may have an equal or even higher prevalence than blepharospasm or cervical dystonia.[43] Oromandibular dystonia is considered a rare disorder; however, in reality, several cases are incorrectly diagnosed.

### Etiology

Oromandibular dystonia is part of the clinical spectrum of a wide variety of diseases. The causes of OMD can be idiopathic (unknown cause), inherited (dystonia of proven genetic origin), and acquired (dystonia due to a known specific cause).[2] Numerous cases of focal or segmental isolated dystonia with onset in adulthood are idiopathic. Causes of inherited dystonia include autosomal dominant (DYT-*THAP1*, DYT-*TAF1*, DYT-*ATP1A3*, DYT-*KMT2B*, Huntington's disease, neuroferritinopathy), autosomal recessive (pantothenate kinase-associated neurodegeneration, Wilson's disease, chorea-acanthocytosis, Gaucher's disease), X-linked (Lesch–Nyhan syndrome, McLeod syndrome), and mitochondrial (Leigh syndrome, deafness-dystonia syndrome).[2,4] Causes of acquired dystonia include perinatal brain injury (dystonic cerebral palsy, delayed-onset dystonia), infection (viral encephalitis,

encephalitis lethargica, subacute sclerosing panencephalitis, human immunodeficiency virus (HIV) infection, tuberculosis, and syphilis), drugs (levodopa and dopamine agonists, neuroleptics, anticonvulsants, and calcium channel blockers), toxic (manganese, cobalt, carbon disulfide, cyanide, methanol, disulfiram, and 3-nitropropionic acid), vascular (ischemia, hemorrhage, arteriovenous malformation), neoplastic (brain tumor, paraneoplastic encephalitis), brain injury (head trauma, brain surgery, and electrical injury), and functional (psychogenic).[2,4]

The pathophysiology of dystonia has not been fully elucidated. Dystonia was originally considered a basal ganglia disorder; however, it has been postulated to be a network disorder involving not only the basal ganglia but also the entire motor system, including the premotor and motor cortex, and the sensory system and thalamus. In addition, the cerebellum, which is directly connected to the basal ganglia, plays a significant role.[45,46] An autopsy study identified pathological markers in the striatum and cerebellum.[47] The pathophysiology of OMD also remains unknown.[48] Cortical negative shifts before voluntary movements, known as movement-related cortical potentials (MRCPs), reflect central motor control processes.[48–50] Reduced amplitude of MRCPs has been reported in other types of dystonia such as cervical dystonia or writer's cramp.[48] A study comparing MRCPs between patients with OMD and normal participants found significantly reduced amplitudes of MRCPs over central and parietal areas for mouth opening and lateral movements in OMD patients, suggesting impaired cortical preparatory processes for mandibular movements.[48] Further research with a larger sample is needed to elucidate the pathophysiology of OMD.

Injury to the peripheral nervous system has been associated with various movement disorders, including dystonia, hemifacial spasm, tremors, myoclonus, tics, and parkinsonism.[51–54] Although no confirmatory test exists to determine whether a movement disorder is genuinely induced by peripheral injury,[53] even minor alterations in normal anatomy or physiology following dental procedures may result in peripherally induced movement disorders in predisposed patients.[54]

### Treatment

If neurological diseases such as Parkinson's disease have already been diagnosed and treated in a patient with OMD, OMD should be treated simultaneously by attending physicians.[1,8] However, if a neurological disease is suspected but not yet diagnosed, the patient should be referred to specialists.[1,8] Similarly, collaboration with a psychiatrist is necessary for the treatment of tardive dystonia.

The treatment of OMD must be multimodal and individualized, and current methods include pharmacological,[4,8,38] botulinum toxin,[1,8,32,55–57] muscle afferent block,[58,59] occlusal splint (sensory trick splint),[41] and surgical therapies (coronoidotomy).[34–36] Deep brain stimulation has been increasingly applied in patients with other types of dystonia. However, patients with OMD have responded unsatisfactorily.[60] Chemodenervation with botulinum toxin, that is, botulinum toxin therapy, is considered the first-line treatment for OMD. Botulinum toxin therapy methods for OMD or other movement disorders or conditions have already been reported in detail.[1,33,55–57] Due to the number of muscle spindles, muscle afferent block therapy is more effective for jaw closing dystonia than for jaw opening dystonia.[58,59] A sensory trick splint is especially successful in patients with hyperactivity of the jaw closing muscles. In one study, 83.7% of the responders with splints presented with jaw closing dystonia.[41] Coronoidotomy is only indicated for the most severe type of jaw closing dystonia associated with extremely limited mouth opening.[35,36] However, ⅓ of patients who underwent operation required additional botulinum toxin injections into the masseter and/or medial pterygoid muscles.[36]

## Functional stomatognathic movement disorders (FSMDs)

Functional stomatognathic movement disorders are part of a spectrum of functional neurological disorders and are among the most common causes of neurological disability.[61] The term "functional" is more commonly used than "psychogenic".[62] These disorders are considered to be caused by a complex interplay of biopsychosocial vulnerabilities triggered by psychosocial and/or physical factors.[63] Functional movement disorders often have characteristic clinical features, particularly in the orofacial region.[64] Therefore, a diagnosis should rely not on the exclusion of organic diseases or the presence of psychological symptoms but on the observation of characteristic clinical features.[63,65] Misdiagnosis of FSMDs as awake bruxism or a psychogenic disease is frequent.[66]

### Presentation

In a previous study,[66] a 10-item set of inclusion criteria for FSMDs was formulated based on previously reported criteria for functional movement disorders[63,65] or clinical features in facial functional dystonia,[64,66] aimed at comprehensively assessing 58 patients (42 women, 16 men; mean age: 46.2 years) with FSMDs. The criteria comprised 10 symptoms, with the prevalence of each as follows: rapid onset (74.1%); static course (60.3%); paroxysmal symptoms (86.2%); spreading to multiple sites (89.7%); spontaneous remission (58.6%); inconsistent symptoms (93.1%); distractibility (67.2%); incongruous symptoms (91.4%); the lack of sensory tricks (81.0%); and suggestibility (63.8%).[65] The characteristic and distinguishable features of FSMDs included rapidly repeating lateral or tapping jaw and tongue movements (27.6%), which fluctuated considerably in speed and direction.[66]

The most prevalent complaint of FSMDs was muscle pain (50%).[66] Depression (38%), dysarthria (27.6%), and masticatory disturbances (15.5%) were also observed.[66] Although specific electrophysiological tests or a gold standard for diagnosis are absent, functional movement disorders should be diagnosed with clinical certainty based on the available criteria.[65] Functional movement disorders often exhibit distinctive clinical features in the orofacial region, such as tonic spasms involving the lip, eyelids, perinasal region, and forehead.[64,66] The most common phenotype is tonic jaw deviation involving ipsilateral downward and lateral lip pulling, observed in 84.3% of patients with facial functional movement disorders.[64] Uni- or bilateral orbicularis oculi and platysma contraction are also frequently associated.[63] Common patterns of FSMDs included jaw deviation (74.1%), jaw closing (50%), lip pulling (34.5%), and tongue movement (31%).[65] The classic phenotype (unilateral lip pulling and jaw deviation) was observed in 44.8% of patients (Fig. 2A).[66] Characteristic features of FSMDs, such as repeated rapid jaw (lateral or tapping) and/or tongue movements, were observed in 22.4% of patients (Fig. 2B).[67]

### Epidemiology

The most common presentations of functional movement disorders are tremors, dystonia, myoclonus, and gait disturbance.[63,65] These symptoms were observed in 5–20% of patients in a movement disorder clinic, with functional dystonia being particularly prevalent.[68] Functional neurological disorders have an estimated prevalence of 50 per 100,000 population based on a community registry.[61] Functional movement disorders affecting the orofacial region are more prevalent in women (91.8% in one study[64] and 72.4% in another[66]). The mean age at onset across these two studies was 37 years[64] and 46.2 years,[66] respectively. In one study, out of 1,720 patients with complaints of involuntary movements or muscle contractures in the masticatory, lingual, and/or lower facial muscles, 58 were diagnosed with FSMDs.[66] Therefore, the prevalence of this condition may be relatively low.

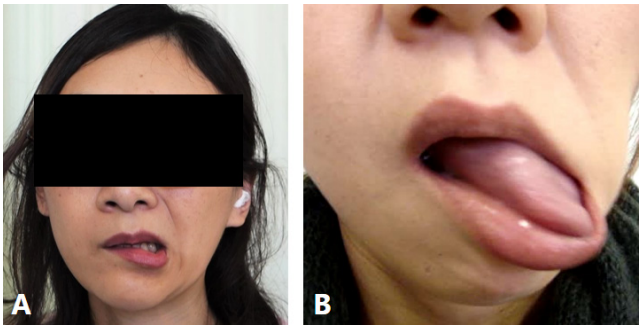


Fig. 2. Clinical presentation of involuntary movements in a patient with a functional stomatognathic movement disorder (FSMD)

A case of the well-known orofacial functional movement disorder phenotype. The patient exhibits unilateral lower lip pulling, jaw deviation and platysma contractions (A). The tongue movement accompanying the mandibular movement is complex and bizarre (B).
Reproduced from reference [64] with permission from the publisher.

### Etiology

The etiology of functional movement disorders is likely multifactorial. Emerging data suggest that regional blood flow and activation patterns on positron emission tomography and functional magnetic resonance imaging are impaired in patients with FSMDs.[69] Neurobiological abnormalities include hypoactivation of the supplementary motor area and abnormal connectivity with areas involved in movement selection or inhibition.[63]

Frequent precipitating events in patients with FSMDs included dental treatment (44.8%) and physical trauma (12.1%).[66] Injury to the oral region or changes in anatomy or physiology following trauma or dental procedures may lead to FSMDs. However, the underlying mechanisms remain unclear.

### Treatment

The first step in treating functional movement disorders should be explaining the diagnosis and confirming patient understanding.[63] Various kinds of treatment include antidepressants, psychological therapy, cognitive behavioral therapy, and transcranial magnetic stimulation.[63,65,69] Depression, anxiety, and pain may be treated pharmacologically. Based on a recent survey of members of the International Parkinson's and Movement Disorder Society, the most effective therapeutic options are avoiding iatrogenic harm and educating patients about their diagnoses.[70]

In a study of 58 patients with FSMDs, symptomatic therapies for presenting symptoms included medication, muscle afferent block therapy, botulinum toxin therapy, and occlusal splint use.[66] When patients with FSMDs showed obvious muscle hyperactivity, botulinum toxin therapy successfully improved symptoms.[66] For typical cases of jaw or tongue deviation, botulinum toxin should be injected into the lateral pterygoid[33] or tongue muscles.[57] Additional targeted muscles may include the platysma, orbicularis oris, risorius, mentalis, and depressor anguli oris muscles.[33] Occlusal splints occasionally prove effective for patients experiencing a sensory trick in the oral cavity.[41] When therapies resulted in ineffective responses, patients were referred to psychiatrists or movement disorder experts for potential cognitive behavior therapy[71] or physiotherapy.[72]

## Hemimasticatory spasm (HMS)

Hemimasticatory spasm is characterized by intermittent paroxysmal contractions of the unilateral jaw closing muscles, resulting in brief twitches and/or prolonged spasms.[73–77]

## Presentation

Involuntary movements can cause masticatory muscle pain, tongue or cheek biting, and temporomandibular joint dislocation,[73,78,79] leading to masticatory disturbances or dysarthria. Hemimasticatory spasm is occasionally accompanied by hemifacial atrophy or localized scleroderma.[76–78,80] Scleroderma is a chronic connective tissue disease that manifests with skin lesions. A recent comprehensive review of HMS patients reported involvement of the masseter (97.4%), temporalis (48.3%), medial pterygoid (6%), and tongue (1.7%) muscles.[81] Common triggers precipitating spasms include chewing, talking, and laughing.[81] Brief twitches and often painful spasms can last from a few seconds to minutes.

## Epidemiology

However, the prevalence of HMS has not yet been reported. It is assumed that the majority of patients without significant hemifacial atrophy or scleroderma visited dentists or dental surgeons and were diagnosed with bruxism. Thus, the actual prevalence of HMS may be much higher than expected. A recent comprehensive review reported that the mean age at onset was 37.1 years. Hemimasticatory spasm occurred more frequently in women (61.5%) than in men (38.5%).[81]

## Etiology

However, the underlying mechanisms of HMS remain unclear. Linear scleroderma can involve the lower face (Parry–Romberg syndrome) and the upper face (en coup de sabre), named for its resemblance to "a stroke from a sword".[82] Deep tissue changes resulting from linear scleroderma can cause localized injury to the motor fibers of the trigeminal nerve.[76,79] Parry–Romberg syndrome is a rare craniofacial disorder characterized by progressive hemiatrophy of the skin, subcutaneous tissue, fat, and, in severe cases, underlying muscle, cartilage, and bone.[82] There is a reported close relationship between Parry–Romberg syndrome and linear scleroderma en coup de sabre.[83] Parry–Romberg syndrome and scleroderma complicated 17.9% and 23.9% of patients with HMS, respectively.[81] Deep tissue changes from Parry–Romberg syndrome or scleroderma may lead to compression and focal demyelination of the motor branch of the trigeminal nerve.[74,77] Several cases of HMS have been reported following severe dental inflammation.[72] Recent reports indicate HMS onset after dental or oral surgical treatments.[81] Some cases of HMS worsened during pregnancy but improved after childbirth.[84,85] Pregnancy-related hormonal changes may influence the mechanism underlying HMS.

The EMG data of HMS showed irregular bursts of motor unit potentials correlated with twitches or spasms. Individual motor unit potentials showed high-frequency activity, up to 200 Hz, suggesting a peripheral origin of the abnormal activity.[73] Unlike unilateral OMD, HMS does not exhibit a co-contraction or overflow phenomenon. Masseter reflexes were either absent or delayed in all examined patients. A distinctive electrophysiological finding of HMS was the absence of silent periods during spasms (Fig. 3). Complete efferent block to the muscles is an exceptional and unique finding in HMS.[76] Based on these electrophysiological findings, HMS is hypothesized to originate from either the motor root or the motor nucleus of the trigeminal nerve.[74–76]

## Treatment

There are several treatment modalities available for patients with HMS. Surgical interventions, such as microvascular decompression, may be effective in definitively diagnosing patients with vascular compression.[86,87] Botulinum toxin therapy for jaw closing muscles is highly effective in improving spasms in patients with HMS. However, repeated injections of botulinum toxin may cause facial hemiatrophy due to masseter muscle atrophy and masticatory disturbance from reduced bite force.[1,34] As an alternative, muscle afferent block therapy blocks muscle afferents for the treatment of focal dystonia,[88] and intramuscular injection of lidocaine reduces the efficacy of muscle spindle afferents without causing unwanted weakness.[58,59] Involuntary HMS contractions persist during sleep, causing some patients to stay awake due to contractions and related pain. Therefore, inserting an occlusal splint during sleep can be beneficial for patients with HMS. Opening the mouth stretches jaw closing muscles, thereby affecting signals from the muscle spindles.[81] Increasing the occlusal vertical dimension with a splint may slightly stretch jaw closing muscles, making spasms less likely to occur.[81] When a clear cause has been identified, such as vascular compression of trigeminal nerve motor roots, surgical procedures such as microvascular decompression can be beneficial for patients.[86,87]

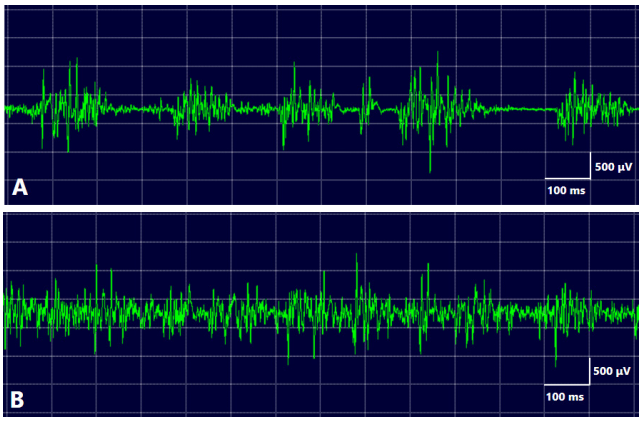


Fig. 3. Electromyography (EMG) of the masseter muscle in a patient with hemimasticatory spasm (HMS)

Several irregular brief bursts of multiple motor unit potentials were observed in the masseter muscle (A). No silent period was observed during prolonged spasms (B).

# Differential diagnosis of involuntary movements

Characteristic clinical findings for the differential diagnosis of movement disorders of the stomatognathic system are summarized in Table 1.

## Oromandibular dystonia (OMD)

Among the 6 subtypes of OMD (Fig. 1), tongue dystonia, jaw opening dystonia, and lip dystonia are rarely diagnosed as bruxism. These patients are often suspected to have psychiatric disorders and are referred to psychiatrists. Jaw closing, deviation, and protrusion dystonia are often diagnosed as awake bruxism if associated with jaw closure, clenching, and grinding. Jaw closing dystonia accounts for more than 60% of OMD cases and is the most frequent subtype.[1,6–8] According to the current definition of bruxism,[9] approx. 70% of OMD patients may be diagnosed with bruxism. Among the aforementioned characteristic clinical features of OMD, task specificity and sensory tricks are less likely to occur over long periods after OMD onset. In other words, symptoms may occur only during conversations in the early stages of OMD onset, but abnormal muscle contractions gradually persist during eating and waking hours.[1,7] Stereotypy and morning benefits are often relatively preserved. Although OMD symptoms subside during sleep, sleep bruxism can coexist with other movement disorders in some patients. Careful differential diagnosis between OMD and awake bruxism is essential for proper treatment.

## Functional stomatognathic movement disorders (FSMDs)

Most patients with FSMDs are suspected to have psychiatric disorders and are often referred to psychiatrists. Common patterns observed in patients with FSMDs include jaw deviation, jaw closing, lip pulling, and tongue movement.[66] When a patient exhibits jaw closing, they may be diagnosed with awake bruxism. According to the latest definition, approx. half of FSMD patients may be diagnosed with awake bruxism.[11] Functional movement disorder presents with various clinical features, including inconsistent or incongruous symptoms, spreading to

**Table 1.** Summary of characteristic features for a differential diagnosis of movement disorders in the stomatognathic system

| Feature | Bruxism | | OMD | FSMDs | HMS |
|---|---|---|---|---|---|
| | sleep bruxism | awake bruxism | | | |
| Involuntary muscle contraction during sleep | often during a period of arousal | no contraction | no contraction | no contraction | persists during sleep |
| Symptoms upon awaking | the worst muscle symptoms in many cases | not present, gradual worsening over time | absent or mild, gradual worsening over time | no temporal change | no temporal change |
| Involved muscles | masticatory muscles | | masticatory and lingual muscles, including the muscles in the stomatognathic system | masticatory and lingual muscles, including the muscles in the stomatognathic system | masseter, temporalis, rarely medial pterygoid |
| Age at onset | from childhood to their 20s and 30s | | between their 40s and 60s<br>mean age: 51 years[7,40] | between their 40s and 60s<br>mean age: 46.2 years[64] | between their 20s and 40s<br>mean age: 37.1 years[79] |
| Gender preponderance | equal prevalence | F (57%)[24] | F (63–66%)[7,40] | F (72.4%)[64] | F (61.2%)[79] |
| Estimated prevalence per 100,000 people | 1,000–15,000[22] | 22,000–30,000[22] | 9.8[41] | uncertain | uncertain |
| Effectiveness of the occlusal splint | often effective | effective in few cases | effective in some cases with jaw closing dystonia[39] | effective in few cases[64] | effective in few cases[79] |
| Other characteristic features | the indentations of the lip or tongue<br>tooth wear<br>linea alba<br>the exostoses/tori of the alveolar bone | | stereotypy (95.8%)[7]<br>task specificity (69.9%)[7]<br>sensory tricks (51.4%)[7]<br>the morning benefit (47.3%)[7] | inconsistent symptoms (93.1%)[64]<br>incongruous symptoms (91.4%)[64]<br>spreading to multiple sites (89.7%)[64]<br>paroxysmal symptoms (86.2%)[64]<br>the lack of sensory tricks (81.0%)[64]<br>a rapid onset (74.1%)[64]<br>distractibility (67.2%)[64]<br>suggestibility (63.8%)[64]<br>a static course (60.3%)[64]<br>spontaneous remission (58.6%)[64] | unilaterality (98.3%)[79]<br>hemifacial atrophy (27.4%)[79]<br>the lack of a silent period (23.9%)[79]<br>morphea or scleroderma (23.9%)[79]<br>Parry–Romberg syndrome (17.9%)[79]<br>the lack of co-contraction<br>the lack of the overflow phenomenon |

OMD – oromandibular dystonia; FSMD – functional stomatognathic movement disorder; HMS – hemimasticatory spasm; F – female.

multiple sites, and paroxysmal symptoms.[66] The FSMD patients should be carefully examined and, if suspected, referred to a psychiatrist or a specialist in involuntary movements.

### Hemimasticatory spasm (HMS)

The differential diagnosis of HMS from bruxism is problematic. All patients diagnosed with HMS by the author had previously received a bruxism diagnosis from dentists or oral surgeons.[81] Involuntary movements mostly disappear during sleep; however, HMS symptoms persist, and associated pain can occasionally awaken patients. Patients are often suspected of having bruxism and seek dental care. Despite being considered a rare disorder, nearly all patients with HMS are initially diagnosed and treated for bruxism by dental professionals.[81] The distinguishing features of HMS include unilateral symptoms (98.3%), morphea or scleroderma, and unilateral facial atrophy (Table 1). A notable finding in HMS is the absence of a silent period on EMG (Fig. 3B). When HMS is suspected, EMG should be performed for differential diagnosis, and referral to a neurosurgeon is recommended for a comprehensive evaluation to identify potential vascular compression of the trigeminal nerve motor root. Vascular decompression surgery can provide complete relief from symptoms.[86,87]

## Proposals for future studies

Numerous dental clinicians and researchers have been interested in bruxism for more than half a century, and many related studies have been published. A PubMed search revealed nearly 5,000 articles on bruxism and approx. 800 review articles. Additionally, the number of published papers has increased rapidly in recent years. Many excellent papers have been published that took a multifaceted approach and studied bruxism in terms of arterial hypertension,[89] genetic basis,[90] metabolic and hormonal disturbances,[91] sleep structure,[92] serotonin pathway,[93] the relationship between temporomandibular disorders,[94] and rhythmic masticatory muscle activity clusters,[95] but many focusing on sleep bruxism. Studies on awake bruxism, although increasing in number, are lacking. Despite researchers working on bruxism for a long time, there have been no significant developments in treatment methods, and various clinical issues remain inconclusive. One reason for this may be that the definition or diagnostic criteria for bruxism are unclear, and other involuntary movements may be diagnosed as bruxism. According to the current definition, all cases of jaw closing dystonia and HMS, as well as some cases of FSMDs or oral dyskinesia, can be diagnosed as bruxism.[11] Furthermore, because bruxism may be diagnosed if there is bracing or thrusting, even without tooth contact,[11] several other movement disorders can also be classified as bruxism. Involuntary movements other than HMS and palatal tremors disappear during sleep. Therefore, involuntary movements are less frequently diagnosed as sleep bruxism and more frequently as awake bruxism. In the current definition, various jaw muscle activities without tooth contact are included under the umbrella term "bruxism".[20] With this definition, because there is no cut-off point, muscle activity after the end of sleep apnea[96,97] is also considered a potential protective factor, and masticatory muscle activities are considered not only as disorders but also as behaviors.[11] Several healthy individuals without clinical problems can be diagnosed with bruxism. The author believes that the diagnostic criteria need to be stricter, and exclusion criteria should be added to rule out other involuntary movements. Otherwise, it may be necessary to distinguish between primary and secondary bruxism and redefine both terms. Conti et al. concludes that the absence of an adequate definition of bruxism, the nondistinction between circadian manifestations, and the use of various measurement techniques found in several studies preclude any solid and convincing conclusions on the existence of "secondary" bruxism.[98] If the experimental group in a study included patients with bruxism with other involuntary movements or healthy individuals whose etiology was completely different from that of primary bruxism, the probability of not obtaining statistically significant results would increase. It has been postulated that a stricter definition of bruxism would provide more reliable results.

The Standardized Tool for the Assessment of Bruxism (STAB) was developed to provide a multidimensional evaluation of bruxism status, comorbid conditions, etiology, and consequences.[99,100] The STAB contains only one questionnaire on orofacial motor disorders in Axis B with the following question: "Have you been diagnosed with or do you suffer from possible signs of one of the following conditions?" Examples of orofacial motor disorders include orofacial dyskinesia, OMD, Parkinson's disease, Tourette syndrome, hemifacial spasms, and tardive dyskinesia. Patients with Rett syndrome, FSMDs, or HMS were excluded. Hemifacial spasms are often caused by compression of the facial nerve by blood vessels, leading to involuntary contraction of the orbicularis oculi and/or facial muscles. Hemifacial spasms are rarely associated with bruxism. As mentioned above, movement disorders of the stomatognathic system are unlikely to be correctly diagnosed, and an examiner must diagnose orofacial motor disorders that the questionnaire may miss. Experts in bruxism may make such a diagnosis; however, this is difficult for most dental clinicians. In addition, the bruxism screener (BruxScreen), developed for large-scale epidemiological research projects and general dental practice, does not include questions regarding movement disorders.[101] Therefore, other involuntary movements that cause mouth closure, clenching, and grinding may also

be classified as bruxism. Furthermore, a common rating scale is required to objectively evaluate bruxism symptoms and changes after treatment. The latest definition of bruxism includes no cut-off point, and masticatory muscle activities are considered not only as disorders but also as behaviors; therefore, a rating scale may be considered unnecessary. Rating scales specific to each type of dystonia, such as cervical dystonia and blepharospasm, have been developed. A rating scale for OMD has also been developed, and its reliability and validity have been verified and applied clinically.[6,55]

Involuntary movements of the stomatognathic system are considered a blind spot in medicine and dentistry. Several patients with these conditions spend years transferring between medical and dental departments. Most dental professionals focus only on bruxism and have very little knowledge of, and may be indifferent to, the other involuntary movements discussed in this review. Conversely, many medical professionals, particularly neurologists specializing in involuntary movements, are not interested in the involuntary movements of the oral cavity. Problems arise when medical professionals administer botulinum toxin to the masticatory muscles, especially the lateral pterygoid muscle.[1] Several patients with involuntary movement from the United States and Europe visit our outpatient clinic for treatment. Although some of the world's most famous experts on involuntary movements can diagnose movement disorders in the oral region, they often cannot identify which masticatory muscles are involved or administer botulinum toxin injections. Therefore, several patients visit our department at their own expense for airfare, accommodation, and treatment. The diagnosis and treatment of involuntary movements in the stomatognathic system require cooperation between medical professionals (neurologists, neurosurgeons, and otorhinolaryngologists) and dental professionals (dentists, oral surgeons, temporomandibular joint specialists, and prosthodontists). Dental and medical professionals should take an interest in movement disorders of the stomatognathic system. In the 1990s, Lavigne et al. applied polysomnography, a sleep medicine method, to sleep bruxism studies, resulting in dramatic advances in the study of sleep bruxism.[29–30] Although research and clinical management of awake bruxism seem to require knowledge and experience regarding movement disorders, the international expert panel currently working mainly on the definition of bruxism does not seem to include movement disorder experts. Clinicians are often unaware that they are diagnosing and treating patients with a variety of involuntary movements as bruxism. When a patient ceases treatment with a clinician, the clinician may assume that the symptoms have subsided. However, in several cases, they often seek care and visit a neurologist or neurosurgeon because treatments for bruxism often prove ineffective. Nevertheless, even neurologists and neurosurgeons, unless they specialize in involuntary movements, may encounter challenges in accurately diagnosing and effectively treating these patients with involuntary movements in the oral region. The author hopes that a multidisciplinary team approach will be possible in many hospitals in various countries, where medical and dental professionals are interested in involuntary movements in the oral region and can collaborate to diagnose and treat them.[102,103] The author believes that this would benefit wandering patients with movement disorders in the stomatognathic system and patients who are not properly diagnosed and treated. It could also lead to rapid advancements in clinical practice and research on involuntary movements in the oral region.

## Conclusions

Each movement disorder has its characteristic clinical features: OMD – task specificity, sensory tricks and the morning benefit; FSMDs – inconsistent and incongruous symptoms, spreading to multiple sites and the lack of sensory tricks; and HMS –the paroxysmal contraction of unilateral jaw closing muscles, the persistence of symptoms during sleep and the loss of a silent period. A careful differential diagnosis is essential for adequate and effective treatment of involuntary movements. Refining the definition of bruxism may be necessary to prevent involuntary movements from being diagnosed as bruxism. The movement disorders of the stomatognathic system should be diagnosed and treated using a multidisciplinary approach.

## Ethics approval and consent to participate

Not applicable.

## Data availability

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

## Consent for publication

Not applicable.

### ORCID iDs

Kazuya Yoshida https://orcid.org/0000-0002-2985-4347

### References

1. Yoshida K. Botulinum toxin therapy for oromandibular dystonia and other movement disorders in the stomatognathic system. *Toxins (Basel)*. 2022;14(4):282. doi:10.3390/toxins14040282
2. Albanese A, Bhatia K, Bressman SB, et al. Phenomenology and classification of dystonia: A consensus update. *Mov Disord*. 2013;28(7):863–873. doi:10.1002/mds.25475
3. Ella B, Ghorayeb I, Burbaud P, Guehl D. Bruxism in movement disorders: A comprehensive review. *J Prosthodont*. 2017;26(7):599–605. doi:10.1111/jopr.12479

4. Saraf U, Chandarana M, Divya KP, Krishnan S. Oromandibular dystonia – a systematic review. *Ann Indian Acad Neurol*. 2022;25(1):26–34. doi:10.4103/aian.aian_242_21
5. Yoshida K. Multilingual website and cyberconsultations for oromandibular dystonia. *Neurol Int*. 2018;10(1):7536. doi:10.4081/ni.2018.7536
6. Yoshida K. Development and validation of a disease-specific oromandibular dystonia rating scale (OMDRS). *Front Neurol*. 2020;11:583177. doi:10.3389/fneur.2020.583177
7. Yoshida K. Oromandibular dystonia screening questionnaire for differential diagnosis. *Clin Oral Investig*. 2019;23(1):405–411. doi:10.1007/s00784-018-2449-3
8. Yoshida K. Therapeutic strategies for oromandibular dystonia [in German]. *Fortschr Neurol Psychiatr*. 2021;89(11):562–572. doi:10.1055/a-1375-0669
9. Yoshida K. Etiology of pneumoparotid: A systematic review. *J Clin Med*. 2022;12(1):144. doi:10.3390/jcm12010144
10. Yoshida K. Superior dislocation of the mandibular condyle into the middle cranial fossa: A comprehensive review of the literature. *J Clin Med*. 2023;12(11):3781. doi:10.3390/jcm12113781
11. 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
12. Chattrattrai T, Janal MN, Lobbezoo F, Raphael KG. The association between sleep bruxism and awake bruxism: Polysomnographic and electromyographic recordings in women with and without myofascial pain. *J Oral Rehabil*. 2023;50(9):822–829. doi:10.1111/joor.13468
13. Lavigne GJ, Manzini C, Kato T. Sleep bruxism. In: Kryger MH, Roth T, Dement WC, eds. *Principles and Practice of Sleep Medicine*. 4th ed. Philadelphia, PA: Elsevier Saunders; 2005:946–959.
14. Lavigne G, Kato T, Herrero Babiloni A, et al. Research routes on improved sleep bruxism metrics: toward a standardised approach. *J Sleep Res*. 2021;30(5):e13320. doi:10.1111/jsr.13320
15. Thymi M, Lobbezoo F, Aarab G, et al. Signal acquisition and analysis of ambulatory electromyographic recordings for the assessment of sleep bruxism: A scoping review. *J Oral Rehabil*. 2021;48(7):846–871. doi:10.1111/joor.13170
16. Bracci A, Djukic G, Favero L, Salmaso L, Guarda-Nardini L, Manfredini D. Frequency of awake bruxism behaviours in the natural environment. A 7-day, multiple-point observation of real-time report in healthy young adults. *J Oral Rehabil*. 2018;45(6):423–429. doi:10.1111/joor.12627
17. Bracci A, Lobbezoo F, Häggman-Henrikson B, et al. Current knowledge and future perspectives on awake bruxism assessment: Expert consensus recommendations. *J Clin Med*. 2022;11(17):5083. doi:10.3390/jcm11175083
18. Colonna A, Bracci A, Ahlberg J, et al. Ecological momentary assessment of awake bruxism behaviors: A scoping review of findings from smartphone-based studies in healthy young adults. *J Clin Med*. 2023;12(5):1904. doi:10.3390/jcm12051904
19. Bucci R, Manfredini D, Lenci F, Simeon V, Bracci A, Michelotti A. Comparison between ecological momentary assessment and questionnaire for assessing the frequency of waking-time non-functional oral behaviours. *J Clin Med*. 2022;11(19):5880. doi:10.3390/jcm11195880
20. Bracci A, Lobbezoo F, Colonna A, et al. Research routes on awake bruxism metrics: implications of the updated bruxism definition and evaluation strategies. *J Oral Rehabil*. 2024;51(1):150–161. doi:10.1111/joor.13514
21. Pereira NC, Oltramari PV, Conti PC, et al. Frequency of awake bruxism behaviour in orthodontic patients: Randomised clinical trial: Awake bruxism behaviour in orthodontic patients. *J Oral Rehabil*. 2021;48(4):422–429. doi:10.1111/joor.13130
22. Asami K, Fujisawa M, Saito-Murakami K, et al. Assessment of awake bruxism – combinational analysis of ecological momentary assessment and electromyography. *J Prosthodont Res*. 2024;68(1):166–171. doi:10.2186/jpr.JPR_D_22_00289
23. American Academy of Sleep Medicine (AASM). *International Classification of Sleep Disorders, Revised: Diagnostic and Coding Manual*. Chicago, IL; 2001.
24. Melo G, Duarte J, Pauletto P, et al. Bruxism: An umbrella review of systematic reviews. *J Oral Rehabil*. 2019;46(7):666–690. doi:10.1111/joor.12801
25. Khoury S, Carra MC, Huynh N, Montplaisir J, Lavigne GJ. Sleep bruxism-tooth grinding prevalence, characteristics and familial aggregation: Aa large cross-sectional survey and polysomnographic validation. *Sleep*. 2016;39(11):2049–2056. doi:10.5665/sleep.6242
26. Wetselaar P, Vermaire EJ, Lobbezoo F, Schuller AA. The prevalence of awake bruxism and sleep bruxism in the Dutch adult population. *J Oral Rehabil*. 2019;46(7):617–623. doi:10.1111/joor.12787
27. 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
28. Kato T, Rompré P, Montplaisir JY, Sessle BJ, Lavigne GJ. Sleep bruxism: An oromotor activity secondary to micro-arousal. *J Dent Res*. 2001;80(10):1940–1944. doi:10.1177/00220345010800101501
29. Lavigne GJ, Rompré PH, Poirier G, Huard H, Kato T, Montplaisir JY. Rhythmic masticatory muscle activity during sleep in humans. *J Dent Res*. 2001;80(2):443–448. doi:10.1177/00220345010800020801
30. Lavigne GJ, Huynh N, Kato T, et al. Genesis of sleep bruxism: Motor and autonomic-cardiac interactions. *Arch Oral Biol*. 2007;52(4):381–384. doi:10.1016/j.archoralbio.2006.11.017
31. 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
32. 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
33. Yoshida K. How do I inject botulinum toxin into the lateral and medial pterygoid muscles? *Mov Disord Clin Pract*. 2017;4(2):285. doi:10.1002/mdc3.12460
34. Yoshida K. Effects of botulinum toxin type A on pain among trigeminal neuralgia, myofascial temporomandibular disorders, and oromandibular dystonia. *Toxins (Basel)*. 2021;13(9):605. doi:10.3390/toxins13090605
35. Yoshida K. Coronoidotomy as treatment for trismus due to jaw-closing oromandibular dystonia. *Mov Disord*. 2006;21(7):1028–1031. doi:10.1002/mds.20859
36. Yoshida K. Surgical intervention for oromandibular dystonia-related limited mouth opening: Long-term follow-up. *J Craniomaxillofac Surg*. 2017;45(1):56–62. doi:10.1016/j.jcms.2016.10.009
37. Yoshida K. Mouth opening retaining appliance after coronoidotomy for the treatment of trismus: Effects on pain during postoperative training and maximal extent of mouth opening. *Clin Surg*. 2020;5:2737. doi:10.25107/cis-v5-id2737
38. Yoshida K. Involuntary movements of the stomatognathic region. https://sites.google.com/site/oromandibulardystoniaenglish. Accessed January 1 2024.
39. Yoshida K, Iizuka T. Botulinum toxin treatment for upper airway collapse resulting from temporomandibular joint dislocation due to jaw-opening dystonia. *Cranio*. 2006;24(3):217–222. doi:10.1179/crn.2006.035
40. Yoshida K. Botulinum neurotoxin injection for the treatment of recurrent temporomandibular joint dislocation with and without neurogenic muscular hyperactivity. *Toxins (Basel)*. 2018;10(5):174. doi:10.3390/toxins10050174
41. Yoshida K. Sensory trick splint as a multimodal therapy for oromandibular dystonia. *J Prosthodont Res*. 2018;62(2):239–244. doi:10.1016/j.jpor.2017.09.004
42. Slaim L, Cohen M, Klap P, et al. Oromandibular dystonia: Demographics and clinical data from 240 patients. *J Mov Disord*. 2018;11(2):78–81. doi:10.14802/jmd.17065
43. Scorr LM, Factor SA, Parra SP, et al. Oromandibular dystonia: A clinical examination of 2,020 cases. *Front Neurol*. 2021;12:700714. doi:10.3389/fneur.2021.700714
44. Yoshida K. Prevalence and incidence of oromandibular dystonia: An oral and maxillofacial surgery service-based study. *Clin Oral Investig*. 2021;25(10):5755–5764. doi:10.1007/s00784-021-03878-9
45. Neychev VK, Gross RE, Lehéricy S, Hess EJ, Jinnah HA. The functional neuroanatomy of dystonia. *Neurobiol Dis*. 2011;42(2):185–201. doi:10.1016/j.nbd.2011.01.026
46. Shakkottai VG, Batla A, Bhatia K, et al. Current opinions and areas of consensus on the role of the cerebellum in dystonia. *Cerebellum*. 2017;16(2):577–594. doi:10.1007/s12311-016-0825-6

47. Miyamoto R, Sumikura H, Takeuchi T, et al. Autopsy case of severe generalized dystonia and static ataxia with marked cerebellar atrophy. *Neurology*.2015;85(17):1522–1524.doi:10.1212/WNL.0000000000002061
48. Yoshida K, Kaji R, Kohara N, et al. Movement-related cortical potentials before jaw excursions in patients with oromandibular dystonia. *Mov Disord*. 2003;18(1):94–100. doi:10.1002/mds.10296
49. Yoshida K, Kaji R, Hamano T, Kohara N, Kimura J, Iizuka T. Cortical distribution of Bereitschaftspotential and negative slope potential preceding mouth-opening movements in humans. *Arch Oral Biol*. 1999;44(2):183–190. doi:10.1016/s0003-9969(98)00122-8
50. Yoshida K, Kaji R, Hamano T, et al. Cortical potentials associated with voluntary mandibular movements. *J Dent Res*. 2000;79(7):1514–1518. doi:10.1177/00220345000790071401
51. Cardoso F, Jankovic J. Peripherally induced tremor and parkinsonism. *Arch Neurol*. 1995;52(3):263–270. doi:10.1001/archneur.1995.00540270055019
52. Jankovic J. Peripherally induced movement disorders. *Neurol Clin*. 2009;27(3):821–832. doi:10.1016/j.ncl.2009.04.005
53. Lenka A, Jankovic J. Peripherally-induced movement disorders: An update. *Tremor Other Hyperkinet Mov (N Y)*. 2023;13:8. doi:10.5334/tohm.758
54. Yoshida K. Peripherally induced movement disorders in the stomatognathic system after oral surgical or dental procedures. *Oral Maxillofac Surg*. 2024. doi:10.1007/s10006-024-01285-4
55. Yoshida K. Effects of botulinum toxin therapy on health-related quality of life evaluated by the oromandibular dystonia rating scale. *Toxins (Basel)*. 2022;14(10):656. doi:10.3390/toxins14100656
56. Yoshida K. Computer-aided design/computer-assisted manufacture-derived needle guide for injection of botulinum toxin into the lateral pterygoid muscle in patients with oromandibular dystonia. *J Oral Facial Pain Headache*. 2018;32(2):e13–e21. doi:10.11607/ofph.1955
57. Yoshida K. Botulinum neurotoxin therapy for lingual dystonia using an individualized injection method based on clinical features. *Toxins (Basel)*. 2019;11(1):51. doi:10.3390/toxins11010051
58. Yoshida K, Kaji R, Kubori T, Kohara N, Iizuka T, Kimura J. Muscle afferent block for the treatment of oromandibular dystonia. *Mov Disord*. 1998;13(4):699–705. doi:10.1002/mds.870130416
59. Yoshida K, Kaji R, Shibasaki H, Iizuka T. Factors influencing the therapeutic effect of muscle afferent block for oromandibular dystonia and dyskinesia: Implications for their distinct pathophysiology. *Int J Oral Maxillofac Surg*. 2002;31(5):499–505. doi:10.1054/ijom.2002.0291
60. Maezawa H, Hirata M, Yoshida K. Neurophysiological basis of deep brain stimulation and botulinum neurotoxin injection for treating oromandibular dystonia. *Toxins (Basel)*. 2022;14(11):751. doi:10.3390/toxins14110751
61. Carson A, Lehn A. Epidemiology. In: Aminoff MJ, Boller F, Swaab DF, eds. *Handbook of Clinical Neurology*. Vol. 139, 3rd series; Hallet M, Sone J, Carson AJ, eds. *Functional Neurologic Disorders*. Amsterdam, the Netherlands: Elsevier/Academic Press; 2016:47–60.
62. Edwards MJ, Stone J, Lang AE. From psychogenic movement disorder to functional movement disorder: It's time to change the name. *Mov Disord*. 2014;29(7):849–852. doi:10.1002/mds.25562
63. Espay AJ, Aybek S, Carson A, et al. Current concepts in diagnosis and treatment of functional neurological disorders. *JAMA Neurol*. 2018;75(9):1132–1141. doi:10.1001/jamaneurol.2018.1264
64. Fasano A, Valadas A, Bhatia KP, et al. Psychogenic facial movement disorders: Clinical features and associated conditions. *Mov Disord*. 2012;27(12):1544-1551. doi:10.1002/mds.25190
65. Espay AJ, Lang AE. Phenotype-specific diagnosis of functional (psychogenic) movement disorders. *Curr Neurol Neurosci Rep*. 2015;15(6):32. doi:10.1007/s11910-015-0556-y
66. Yoshida K. Clinical characteristics of functional movement disorders in the stomatognathic system. *Front Neurol*. 2020;11:123. doi:10.3389/fneur.2020.00123
67. Kaski D, Bronstein AM, Edwards MJ, Stone J. Cranial functional (psychogenic) movement disorders. *Lancet Neurol*. 2015;14(12):1196–1205. doi:10.1016/S1474-4422(15)00226-4
68. Miyasaki JM, Sa DS, Galvez-Jimenez N, Lang AE. Psychogenic movement disorders. *Can J Neurol Sci*. 2003;30(Suppl 1):S94–S100. doi:10.1017/s0317167100003292
69. Schmerler DA, Espay AJ. Functional dystonia. In: Aminoff MJ, Boller F, Swaab DF, eds. *Handbook of Clinical Neurology*. Vol. 139, 3rd series; Hallet M, Sone J, Carson AJ, eds. *Functional Neurologic Disorders*. Amsterdam, the Netherlands: Elsevier/Academic Press; 2016:235–245.
70. LaFaver K, Lang AE, Stone J, et al. Opinions and clinical practices related to diagnosing and managing functional (psychogenic) movement disorders: Changes in the last decade. *Eur J Neurol*. 2020;27(6):975–984. doi:10.1111/ene.14200
71. Goldstein LH, Chalder T, Chigwedere C, et al. Cognitive-behavioral therapy for psychogenic nonepileptic seizures: A pilot RCT. *Neurology*. 2010;74(24):1986–1994. doi:10.1212/WNL.0b013e3181e39658
72. Nielsen G, Buszewicz M, Stevenson F, et al. Randomised feasibility study of physiotherapy for patients with functional motor symptoms. *J Neurol Neurosurg Psychiatry*. 2017;88(6):484–490. doi:10.1136/jnnp-2016-314408
73. Kaufman MD. Masticatory spasm in facial hemiatrophy. *Ann Neurol*. 1980;7(6):585–587. doi:10.1002/ana.410070614
74. Thompson PD, Obeso JA, Delgado G, Gallego J, Marsden CD. Focal dystonia of the jaw and the differential diagnosis of unilateral jaw and masticatory spasm. *J Neurol Neurosurg Psychiatry*. 1986;49(6):651–656. doi:10.1136/jnnp.49.6.651
75. Auger RG, Litchy WJ, Cascino TL, Ahlskog JE. Hemimasticatory spasm: Clinical and electrophysiologic observations. *Neurology*. 1992;42(12):2263–2266. doi:10.1212/wnl.42.12.2263
76. Cruccu G, Inghilleri M, Berardelli A, et al. Pathophysiology of hemimasticatory spasm. *J Neurol Neurosurg Psychiatry*. 1994;57(1):43–50. doi:10.1136/jnnp.57.1.43
77. Radhakrishnan DM, Goyal V, Shukla G, Singh MB, Ramam M. Hemi masticatory spasm: series of 7 cases and review of literature. *Mov Disord Clin Pract*. 2019;6(4):316–319. doi:10.1002/mdc3.12760
78. Esteban A, Traba A, Prieto J, Grandas F. Long term follow up of a hemimasticatory spasm. *Acta Neurol Scand*. 2002;105(1):67–72. doi:10.1034/j.1600-0404.2002.00119.x
79. Pillai AR, Kumar GJ, Kumar P, Nizar SA, Pendyala SK, Hamid Baig FA. Assessment of the efficacy of TMJ arthroscopy-assisted masseter nerve avulsion on hemimasticatory spasm: An original research. *J Pharm Bioallied Sci*. 2023;15(Suppl 1):S110–S113. doi:10.4103/jpbs.jpbs_430_22
80. Kim HJ, Jeon BS, Lee KW. Hemimasticatory spasm associated with localized scleroderma and facial hemiatrophy. *Arch Neurol*. 2000;57(4):576–580. doi:10.1001/archneur.57.4.576
81. Yoshida K. Hemimasticatory spasm: A series of 17 cases and a comprehensive review of the literature. *Front Neurol*. 2024;15:1377289. doi:10.3389/fneur.2024.1377289
82. Tolkachjov SN, Patel NG, Tollefson MM. Progressive hemifacial atrophy: A review. *Orphanet J Rare Dis*. 2015;10:39. doi:10.1186/s13023-015-0250-9
83. El-Kehdy J, Abbas O, Rubeiz N. A review of Parry–Romberg syndrome. *J Am Acad Dermatol*. 2012;67(4):769–784. doi:10.1016/j.jaad.2012.01.019
84. Cersósimo MG, Bertoti A, Roca CU, Micheli F. Botulinum toxin in a case of hemimasticatory spasm with severe worsening during pregnancy. *Clin Neuropharmacol*. 2004;27(1):6–8. doi:10.1097/00002826-200401000-00004
85. Panda AK, Gopinath G, Singh S. Parry–Romberg syndrome with hemimasticatory spasm in pregnancy: A dystonia mimic. *J Neurosci Rural Pract*. 2014;5(2):184–186. doi:10.4103/0976-3147.131675
86. Wu G, Ouyang J, Zhang Z, Liu R. Observation of effects of different surgical treatments on unilateral masticatory muscle spasm. *World Neurosurg*. 2018;110:e560–e566. doi:10.1016/j.wneu.2017.11.050
87. Li Q, Wang X, Wang L, Chen G. Surgical treatment strategies for hemimasticatory spasms. *Chin Med J (Engl)*. 2022;135(9):1105–1107. doi:10.1097/CM9.0000000000002105
88. Yoshida K. Hemimasticatory spasm treated with muscle afferent block therapy and occlusal splint. *J Mov Disord*. 2024;17(2):230–232. doi:10.14802/jmd.23249
89. 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
90. Wieckiewicz M, Bogunia-Kubik K, Mazur G, et al. Genetic basis of sleep bruxism and sleep apnea – response to a medical puzzle. *Sci Rep*. 2020;10(1):7497. doi:10.1038/s41598-020-64615-y
91. Michalek-Zrabkowska M, Wieckiewicz M, Smardz J, et al. Determination of inflammatory markers, hormonal disturbances, and sleepiness associated with sleep bruxism among adults. *Nat Sci Sleep*. 2020;12:969–979. doi:10.2147/NSS.S268470

92. Wieczorek T, Wieckiewicz M, Smardz J, et al. Sleep structure in sleep bruxism: A polysomnographic study including bruxism activity phenotypes across sleep stages. *J Sleep Res*. 2020;29(6):e13028. doi:10.1111/jsr.13028
93. Smardz J, Martynowicz H, Wojakowska A, et al. Lower serotonin levels in severe sleep bruxism and its association with sleep, heart rate, and body mass index. *J Oral Rehabil*. 2022;49(4):422–429. doi:10.1111/joor.13295
94. Cigdem Karacay B, Sahbaz T. Investigation of the relationship between probable sleep bruxism, awake bruxism and temporomandibular disorders using the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD). *Dent Med Probl*. 2023;60(4):601–608. doi:10.17219/dmp/158926
95. Martynowicz H, Lavigne G, Kato T, et al. A case–control study on the effect of rhythmic masticatory muscle activity (RMMA) clusters on sleep fragmentation and severity of orofacial muscle pain in sleep bruxism. *J Sleep Res*. 2023;20:e14072. doi:10.1111/jsr.14072
96. Yoshida K. Effect of a prosthetic appliance for treatment of sleep apnea syndrome on masticatory and tongue muscle activity. *J Prosthet Dent*. 1998;79(5):537–544. doi:10.1016/s0022-3913(98)70175-1
97. Yoshida K. A polysomnographic study on masticatory and tongue muscle activity during obstructive and central sleep apnea. *J Oral Rehabil*. 1998;25(8):603–609. doi:10.1046/j.1365-2842.1998.00290.x
98. Conti PC, Cunha CO, De Castro F Conti AC, et al. Secondary bruxism: A valid diagnosis or just a coincidental finding of additional masticatory muscle activity? A narrative review of literature. *J Oral Rehabil*. 2024;51(1):74–86. doi:10.1111/joor.13592
99. Manfredini D, Ahlberg J, Aarab G, et al. The development of the Standardised Tool for the Assessment of Bruxism (STAB): An international road map. *J Oral Rehabil*. 2024;51(1):15–28. doi:10.1111/joor.13380
100. Manfredini D, Ahlberg J, Aarab G, et al. Standardised Tool for the Assessment of Bruxism. *J Oral Rehabil*. 2024;51(1):29–58. doi:10.1111/joor.13411
101. Lobbezoo F, Ahlberg J, Verhoeff MC, et al. The bruxism screener (BruxScreen): Development, pilot testing and face validity. *J Oral Rehabil*. 2024;51(1):59–66. doi:10.1111/joor.13442
102. Yoshida K. Bruxism or dystonia: That is the question. *J Oral Maxillofac Surg*. 2023;81(8):935–937. doi:10.1016/j.joms.2023.04.012
103. Yoshida K. Reply: Bruxism or dystonia: That is the question. *J Oral Maxillofac Surg*. 2024;82(4):390–391. doi:10.1016/j.joms.2023.12.004

Research letter

# Development, characterization, and biocompatibility and corrosion analyses of a silver-decorated graphene oxide and chitosan surface coating for titanium dental implants: A preliminary report

Devika Biju[1,A–C], Parkavi Arumugam[1,A–C], Saranya Kannan[1,A–C], Pradeep Kumar Yadalam[1,A–C], Vincenzo Ronsivalle[2,D–F], Marco Cicciù[2,D–F], Giuseppe Minervini[3,D–F]

[1] Department of Periodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India
[2] Department of Biomedical and Biotechnological Sciences, University of Catania, Italy
[3] Multidisciplinary Department of Medical-Surgical and Odontostomatological Specialties, University of Campania "Luigi Vanvitelli", Naples, Italy

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

Dental and Medical Problems, ISSN 1644-387X (print), ISSN 2300-9020 (online) *Dent Med Probl*. 2024;61(4):627–632

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

**Funding sources**
None declared

**Conflict of interest**
None declared

**Acknowledgements**
None declared

Received on January 16, 2024
Reviewed on April 1, 2024
Accepted on April 13, 2024

Published online on August 30, 2024

**Cite as**
Biju D, Arumugam P, Kannan S, et al. Development, characterization, and biocompatibility and corrosion analyses of a silver-decorated graphene oxide and chitosan surface coating for titanium dental implants: A preliminary report. *Dent Med Probl.* 2024;61(4):627–632. doi:10.17219/dmp/187107

**DOI**
10.17219/dmp/187107



## Abstract

**Background.** Dental implants are increasingly favored as a therapeutic replacement option for edentulism. Titanium (Ti), due to its excellent biocompatibility and unique osseointegration properties, is commonly used in dental implants. Various surface modifications have been explored to improve osseointegration outcomes. Graphene oxide (GO) is a promising material with various applications. Chitosan, found in the exoskeleton of crustaceans and in marine algae, has several biomedical applications. Silver (Ag) is another promising antibacterial agent that increases permeability and damages the bacterial cell membrane upon binding.

**Objectives.** The present study applied a novel implant surface coating of Ag-decorated GO and chitosan on Ti implants to promote bone formation. We further analyzed the physiochemical and antibacterial properties of this surface coating.

**Material and methods.** A solution was prepared by mixing 3 mL of 1% chitosan solution with 10 mg of Ag-GO nanoparticles (NPs). Titanium metal was heated to 70–80°C on a hotplate and the solution was applied onto Ti to obtain an adhesive surface coating. The coated implant was further analyzed for surface properties, using scanning electron microscopy (SEM), the energy dispersive X-ray (EDX) analysis, the attenuated total reflectance-Fourier transform infrared (ATR-FTIR) technique, and the biocompatibility and corrosion analyses.

**Results.** The SEM analysis revealed a homogenously spread, rough, fibrillar and porous layer of coating on the metal surface. The EDX and ATR-FTIR analyses confirmed the successful coating of the implant surface with Ag-decorated GO and chitosan layers. The cell culture assay demonstrated excellent biocompatibility of the surface coating. The corrosion analysis showed improved corrosion resistance of the developed implant surface coating.

**Conclusions.** The various analyses of the coating showed ideal properties for improved cell attachment, differentiation and proliferation while maintaining an antimicrobial environment on the implant surface.

**Keywords:** chitosan, graphene, quality of life, silver, titanium

## Introduction

Dental implants are becoming increasingly popular as a therapeutic replacement option for edentulism worldwide.[1,2] Titanium (Ti) is the preferred material due to its excellent biocompatibility and unique property of osseointegration with the surrounding bone. Various risk factors, such as smoking, diabetes, poor plaque control, peri-implant pathogenic microbiota, and iatrogenic factors, significantly affect the success of implants.[3] A meta-analysis found that the prevalence of peri-implantitis was 18.5% at the implant level and 12.8% at the patient level.[4] Despite personalized risk reduction strategies, a portion of the population is still affected by peri-implant diseases. This may be attributed to the underlying genetic makeup of individuals, which may cause differential tissue expression and a relative increase in implant placement globally. However, implant surface modifications have shown positive outcomes with improved osseointegration and long-term success rates.[5]

Graphene oxide (GO) is a promising material with various applications in electronics, biosensing, imaging, drug delivery, cancer treatment, tissue engineering, nanotherapeutics, and implantology. It is a single monomolecular layer of graphite with various oxygen-containing functional groups, such as carboxyl, hydroxyl and epoxide ones. Studies have explored the antibacterial potential of GO, finding that synthesized GO has sharp edges that act as nano-knives and disturb the membrane integrity of microbes, leading to cell death.[6]

Chitosan, a natural biopolymeric polysaccharide found in the exoskeleton of crustaceans and in marine algae, has several biomedical applications. Studies have demonstrated the antimicrobial, antioxidant, anti-inflammatory, and osteogenic properties of chitosan.[7–12] The cationic nature of chitosan is responsible for many of its biological functions, including antimicrobial, hemostatic, wound-healing, and controlled drug release activity.

Silver (Ag) is another promising antibacterial agent that increases permeability and damages the bacterial cell membrane upon binding. Inside the cell, Ag reacts with enzymes, producing reactive oxygen species (ROS) that impair DNA replication and cause cell death.[13] The peri-implant space harbors unique and complex microbiota, and peri-implantitis and peri-implant mucositis have been associated with such microbial species as *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerella forsythia*, *Prevotella intermedia*, bacteroides, and *Filifactor* sp. Incorporating antimicrobial agents into implant surface coatings enables controlling microbial species.[13]

Studies have also shown the osteoinductive properties of Ag nanoparticles (NPs) in stem cells through increasing the expression of alkaline phosphatase (ALP), runt-related transcription factor 2 (RUNX2), bone morphogenetic protein 2 (BMP2), collagen type 1 alpha 1 (COL1A1), osteocalcin (OCN), and osteopontin (OPN).[14]

The present study applied an Ag-decorated GO and chitosan coating to Ti implants, analyzing its physicochemical properties, biocompatibility and corrosion resistance.

## Material and methods

The study was conducted at the Department of Biomaterials, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India, using analytical-grade chemicals purchased from Sigma Aldrich, Merck Group, Darmstadt, Germany. Commercially pure Ti grade 2 plates with dimensions of 20 mm × 15 mm × 2 mm were purchased from Ti Anode Fabricators, Chennai, India.

### Development of the implant surface coating

The development of an Ag-decorated GO and chitosan adhesive surface coating involved dispersing 40 mg of reduced graphene in 100 mL of distilled water, adding 1 mmol of silver nitrate solution and 2 mL of 10 mmol sodium borohydride until a color change from black to grey occurred. The study used 1 mmol of silver nitrate to reduce it to AgNPs, aiming for a biocompatible concentration. The quantity of sodium borohydride was chosen based on molecular calculations. The reduction process continued until the color changed to metallic silver, indicating the complete reduction of metal Ag. The solution was centrifuged at 2,500 rpm for 20 min to obtain a pellet, which was dried to yield 10 mg of Ag-decorated GO NPs. A total of 3 mL of 1% chitosan solution of marine origin was mixed with 10 mg of Ag-GO NPs to obtain the final solution for implant surface coating. Titanium metal was heated to 70–80°C on a hotplate and the solution was added to obtain an adhesive surface coating.

### Material characterization

The Ti surface coating was examined for surface properties under a field-emission scanning electron microscope (FE-SEM), using an accelerating voltage of 3 kV at a resolution of 10 µm. The energy dispersive X-ray (EDX) analysis was performed to characterize the samples chemically, and vibrational modes were confirmed through attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy (Alpha II; Bruker, Billerica, USA).

### Biocompatibility analysis

The developed implant surface coating was cultured with human MG-63 osteoblast-like cells (National Centre for Cell Science (NCCS), Pune, India) to analyze biocompatibility, cell morphology and growth pattern. Bare, non-coated Ti implant samples were used as the control. Dulbecco's Modified Eagle Medium (DMEM) was prepared according to

the manufacturer's instructions. All growth media, supplements and cell stains were purchased from HiMedia Laboratories, Thane, India. The DMEM was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin to support cell growth and prevent contamination. The cryopreserved MG-63 cells were thawed in a water bath at 37°C, following standard protocols. The cells were cultured in the prepared culture medium in T-75 flasks until they reached approx. 80% confluency. They were then seeded onto the surface-coated Ti implant samples at a density of 10,000 cells/cm$^2$ for each independent experiment. The cell-seeded implant samples were placed in cell culture dishes and incubated at 37°C in a humidified atmosphere with 5% $CO_2$. After incubation, the samples were stained with rhodamine B, acridine orange and combination staining methods to analyze cell attachment, spreading and proliferation with the use of a confocal laser scanning microscope (DMi8; Leica Camera, Wetzlar, Germany). The cells were transferred onto glass slides, mounted with DAPI for nuclear counterstaining, and analyzed using the ImageJ software (https://imagej.net/ij). The viability of the MG63 cells on Ti implant surfaces was observed at various time periods (24 h, 48 h, 72 h, 96 h, and 120 h), with the results validated 5 times.

## Corrosion analysis

A Ti implant was dipped in a mixture of Ag-decorated GO and chitosan for 45 min under the open circuit potential (OCP). After stabilization, the impedance analysis was performed with an amplitude of ±10 mV. Impedance spectra were recorded over a frequency range from 0.01 Hz to 200 kHz. The polarization study was conducted from –1 V to +0.05 V at a scan rate of 1 mV/s.

## Statistical analysis

The data was analyzed using IBM SPSS Statistics for Windows (IBM Corp., Armonk, USA), with the unpaired *t* test assessing the biocompatibility of the coating as compared to a bare Ti surface. A *p*-value ≤0.05 was considered statistically significant.

# Results

The SEM analysis revealed a homogeneous, rough, fibrous layer of the surface coating on the metal surface, with microporosities ranging from 0.2 µm to 0.5 µm. The porosities were well interconnected with each other throughout the thickness of the surface coating. The Ag-decorated GO nanostructures appeared enmeshed within the chitosan fibrous layer. Multiple lamellar layers of the surface coating were noticeable. In the EDX analysis, Ti appeared as the predominant constituent at 43.6 wt%, attributed to the base material of the implant. Carbon (C) constituted 29.5 wt% and oxygen (O) 23.1 wt%, indicating successful coating with GO and chitosan. Trace amounts of sulfur (S) at 3.8% were also attributed to chitosan. The ATR-FTIR analysis showed the absorbance peaks correlating to the presence of various functional groups. The absorbance peak at 636 cm$^{-1}$ confirmed the presence of the Ti–O functional group. The peaks at 803 cm$^{-1}$, 915 cm$^{-1}$, 1,178 cm$^{-1}$, and 1,314–1,631 cm$^{-1}$ confirmed the presence of sulfides, amides, ethers, alkenes, alkynes, and aromatic compounds attributed to chitosan. The peaks at 1,178 cm$^{-1}$, 1,314–1,631 cm$^{-1}$ and 2,096 cm$^{-1}$ indicated the presence of ethers, alkenes, alkynes, aromatic compounds, and methylene groups attributed to GO, as shown in Table 1.

The confocal analysis revealed viable cells of osteoblastic lineage that showed homogeneous spread and good adherence to the coated implant surfaces. Multiple layers of cells arranged in a stacking pattern were visible. Staining active cells with well-defined cell organelles and mitochondria was achieved by using rhodamine B. Cells with extending filopodia confirmed the proliferation process. The results indicated viable cells and showed that the developed implant surface coating had no cytotoxic effects. Acridine orange staining showed live cells with viable stained DNA. The results confirmed the absence of cytotoxic effects on the DNA or nuclear material of the cells. Cell multiplication was not affected, as evidenced by cell proliferation. Combination staining further confirmed the homogeneous spread of viable cells with well-marked cell organelles. The viability of MG-63 cells on bare and coated Ti implant surfaces was compared at different time intervals (24 h, 48 h, 72 h, 96 h, and 120 h), using the independent *t* test, with a *p*-value ≤0.05 set as statistically significant. No statistically significant difference in cell viability on the coated vs. bare Ti surfaces was observed at any time point (Fig. 1). The results showed that the developed surface coating was as biocompatible as the bare titanium surface, and could be safely used for in vivo applications.

**Table 1.** Absorbance peaks observed with the developed silver (Ag)-decorated graphene oxide (GO) and chitosan implant surface coating (attenuated total reflectance-Fourier transform infrared (ATR-FTIR) analysis)

| Absorbance peak [cm$^{-1}$] | Bond | Functional group |
|---|---|---|
| 636 | Ti–O | titanium oxide |
| 803 | C–S–C deformation | sulfide |
| 915 | C–S interaction | amide |
| 1,034 | oxyethylene ring | hydroxyl group |
| 1,178 | C–O–C stretching | ether |
| 1,314 | C–C | alkene, alkyne, arene |
| 1,482 | C=C of methylenedioxy | aromatic compounds |
| 1,530, 1,631 | bending of NH | amide |
| 1,720 | – | carboxyl group |
| 2,096 | –$CH_2$ group | methylene group |
| 2,832 | O–H stretching | alcohol |

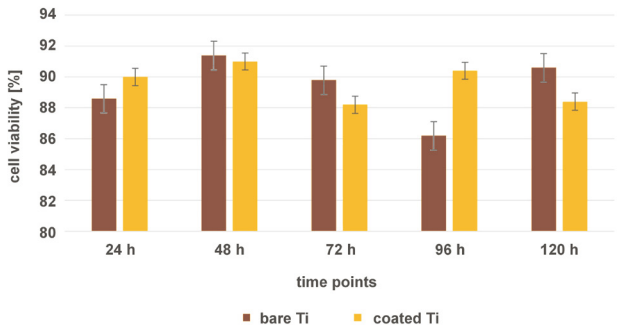


Fig. 1. Comparison of the biocompatibility of bare and coated titanium (Ti) implant surfaces at different time points

In the corrosion analysis, the results showed that the coating had a high corrosion potential (Ecorr) and a low corrosion current density (Icorr) as compared to bare metal. The Nyquist plot showed high charge transfer resistance (RCT), suggesting greater resistance to corrosion. This could be due to the coating preventing corrosive ions from the simulated body fluid from penetrating the metal surface. The Bode impedance value was high at low frequencies, indicating the resistance of the coating. The Bode phase angle was closer to 1, indicating a constant phase element. The corrosion analysis showed that the developed surface coating was more thermodynamically stable with higher impedance than the bare metal surface, which resulted in better corrosion resistance.

## Discussion

The results show the successful coating of the implant surface, which aligns with recent studies on chitosan and GO.[15–18] We chose MG-63 cells due to their similar differentiation capabilities to mesenchymal stem cells, making them popular in osteogenesis studies.[19–21] They provide insights into the cell–material interactions and osseointegration issues. Whereas alternative cell sources and lineages, such as normal human dermal fibroblasts, have been explored for wound healing studies, we found MG-63 cells to be more suitable for osseointegration-related research.

Graphene oxide coatings exhibit good biocompatibility, with beneficial osteogenic effects on MSCs, creating a pro-osteogenic environment through the modulation of the Toll-like receptor (TLR) pathway, and the transforming growth factor beta 1 (*TGF-β1*) and oncostatin M (*OSM*) genes.[22] Studies that analyzed the effect of reduced GO demonstrated its superior ability in the surface adsorption of cells and proteins, promoting osteogenic cell differentiation and proliferation.[23] Graphene oxide has also been shown to accelerate osseointegration and tissue regeneration, as evidenced by increased ALP activity and expression of osteogenesis-related genes in both in vitro and in vivo studies.[23] This property of GO to improve osteogenesis is attributed to its strong proficiency in terms of surface adsorption. The electron cloud of graphene interacts with the hydrophobic segment of serum proteins, thereby improving cell adhesion to the implant surface.

Moreover, the oxygen functional groups in GO further bolster cell adhesion.[24] Graphene oxide-enhanced materials have been shown to improve cell adhesion and osteogenic differentiation by modulating the focal adhesion kinase/p38 (FAK/p38) signaling pathways.[25,26] Additionally, GO has been postulated to exhibit antimicrobial properties through physical and chemical mechanisms.[27] Direct contact with the sharp edges of GO leads to cell death, as the ROS generated through charge transfer cause DNA damage and mitochondrial dysfunction, impairing protein-lipid metabolism. Furthermore, due to its electron transfer capability, it can damage membranes, leading to cell death. A recent systematic review supports these findings, stating that GO-functionalized coatings proved to be a promising solution.[28] Silver NPs may further enhance the antimicrobial properties of GO. This enhanced activity can be attributed to the nanoscale size of Ag, which increases cell membrane permeability and the production of ROS that impair DNA replication, ultimately leading to cell death.[13]

Chitosan in a surface coating further contributes to its antimicrobial properties. Chitosan, a natural biomaterial obtained from the shells of crustacean organisms, exhibits a unique polycationic property that has been postulated to be responsible for antimicrobial activity. The binding of chitosan to the negatively charged cell membrane and its attachment to cellular DNA have been shown to increase cell membrane permeability and affect DNA replication, respectively.[7] Its chelating property has also been shown to affect cell metabolism, causing cell death.[29] Furthermore, studies have demonstrated the osteogenic potential of chitosan.[30–32] It has been proven to increase ALP activity, thereby enhancing calcium (Ca) deposition.

The developed novel implant surface coating with the synergistic combination of Ag-decorated GO and chitosan may contribute to enhanced osteogenic and antimicrobial activity. Cell culture studies prove the optimal biocompatibility of the developed coating. The results of this study suggest that the surface morphology and chemical characteristics of the coating are conducive to improved osteogenesis and antimicrobial action. Based on the preliminary results, it can be concluded that this novel implant surface coating shows promise for further development for clinical applications. In the future, coating the dental implant surface with Ag-decorated GO and chitosan may contribute firstly to improved cell adhesion onto the surface, leading to better cell differentiation. This would ultimately increase the probability of achieving greater secondary stability. Secondly, the antimicrobial properties of the components of the implant coating may help achieve an osteoinductive environment around the implant by preventing the emergence of dysbiotic pathogens that may initiate the disease process. Moreover, the antimicrobial properties of the surface coating may act as an adjunct in the prevention of peri-

implant diseases by compensating for any lapses in the maintenance of good oral hygiene practices. These properties of the developed implant surface coating may clinically translate into improved long-term success of dental implants, potentially paving the way for implant placement in anatomically complicated and systemically compromised cases with greater predictability.

## Limitations

The preliminary results from the study do not directly demonstrate clinical superiority. Further analysis is needed to optimize the thickness, surface tension and wettability of the implant surface coating to achieve positive clinical outcomes. Research on antimicrobial properties, cell line studies and animal studies are needed to evaluate the therapeutic potential of the coating and its impact on peri-implant diseases. Long-term human control trials are essential to validate the clinical efficacy of the coating and to compare it with the clinically proven alternatives.

# Conclusions

The present preliminary report confirmed the successful coating of the implant surface with Ag-decorated GO and chitosan. The various analyses of the coating showed ideal properties for improved cell attachment, differentiation and proliferation while maintaining an antimicrobial environment on the implant surface. The biocompatibility and corrosion resistance of the coating further enhance its value. Further in vitro and in vivo studies should be conducted to explore the potential of this novel coating to achieve improved osseointegration.

## 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

Devika Biju https://orcid.org/0000-0001-9163-1868
Parkavi Arumugam https://orcid.org/0000-0001-5771-8994
Saranya Kannan https://orcid.org/0009-0008-0183-7087
Pradeep Kumar Yadalam https://orcid.org/0000-0002-6653-4123
Vincenzo Ronsivalle https://orcid.org/0000-0003-3059-4981
Marco Cicciù https://orcid.org/0000-0003-2311-9728
Giuseppe Minervini https://orcid.org/0000-0002-8309-1272

## References

1. Ho K, Bahammam S, Chen CY, et al. A cross-sectional survey of patient's perception and knowledge of dental implants in Japan. *Int J Implant Dent*. 2022;8(1):14. doi:10.1186/s40729-022-00410-w
2. Le Thieu MK, Mauland EK, Verket A. Satisfaction and preferences among patients with both implant-supported single crown and tooth-supported fixed dental prosthesis: A pilot study. *Acta Odontol Scand*. 2023;81(5):358–362. doi:10.1080/00016357.2022.2155239
3. Scarano A, Khater AG, Gehrke SA, et al. Current status of peri-implant diseases: A clinical review for evidence-based decision making. *J Funct Biomater*. 2023;14(4):210. doi:10.3390/jfb14040210
4. Rakic M, Galindo-Moreno P, Monje A, et al. How frequent does peri-implantitis occur? A systematic review and meta-analysis. *Clin Oral Investig*. 2018;22(4):1805–1816. doi:10.1007/s00784-017-2276-y
5. Accioni F, Vázquez J, Merinero M, Begines B, Alcudia A. Latest trends in surface modification for dental implantology: Innovative developments and analytical applications. *Pharmaceutics*. 2022;14(2):455. doi:10.3390/pharmaceutics14020455
6. Aunkor MTH, Raihan T, Prodhan SH, Metselaar HS, Fahmida Malik SU, Azad AK. Antibacterial activity of graphene oxide nanosheet against multidrug resistant superbugs isolated from infected patients. *R Soc Open Sci*. 2020;7(7):200640. doi:10.1098/rsos.200640
7. Yilmaz Atay H. Antibacterial activity of chitosan-based systems. In: Jana S, Jana S, eds. *Functional Chitosan*. Springer: Singapore; 2020;457–489. doi:10.1007/978-981-15-0263-7_15
8. Abd El-Hack ME, El-Saadony MT, Shafi ME, et al. Antimicrobial and antioxidant properties of chitosan and its derivatives and their applications: A review. *Int J Biol Macromol*. 2020;164:2726–2744. doi:10.1016/j.ijbiomac.2020.08.153
9. Feng P, Luo Y, Ke C, et al. Chitosan-based functional materials for skin wound repair: Mechanisms and applications. *Front Bioeng Biotechnol*. 2021;9:650598. doi:10.3389/fbioe.2021.650598
10. Yan D, Li Y, Liu Y, Li N, Zhang X, Yan C. Antimicrobial properties of chitosan and chitosan derivatives in the treatment of enteric infections. *Molecules*. 2021;26(23):7136. doi:10.3390/molecules26237136
11. Matica MA, Aachmann FL, Tøndervik A, Sletta H, Ostafe V. Chitosan as a wound dressing starting material: Antimicrobial properties and mode of action. *Int J Mol Sci*. 2019;20(23):5889. doi:10.3390/ijms20235889
12. Pang Y, Qin A, Lin X, et al. Biodegradable and biocompatible high elastic chitosan scaffold is cell-friendly both in vitro and in vivo. *Oncotarget*. 2017;8(22):35583–35591. doi:10.18632/oncotarget.14709
13. Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH. The antibacterial mechanism of silver nanoparticles and its application in dentistry. *Int J Nanomedicine*. 2020;15:2555–2562. doi:10.2147/IJN.S246764
14. Zhang R, Lee P, Lui VCH, et al. Silver nanoparticles promote osteogenesis of mesenchymal stem cells and improve bone fracture healing in osteogenesis mechanism mouse model. *Nanomedicine*. 2015;11(8):1949–1959. doi:10.1016/j.nano.2015.07.016
15. Zhao C, Lu X, Zanden C, Liu J. The promising application of graphene oxide as coating materials in orthopedic implants: Preparation, characterization and cell behavior. *Biomed Mater*. 2015;10(1):015019. doi:10.1088/1748-6041/10/1/015019
16. Kaviya M, Ramakrishnan P, Mohamed SB, et al. Synthesis and characterization of nano-hydroxyapatite/graphene oxide composite materials for medical implant coating applications. *Mater Today Proc*. 2021;36(Pt 2):204–207. doi:10.1016/j.matpr.2020.02.932
17. Murugan N, Murugan C, Sundramoorthy AK. In vitro and in vivo characterization of mineralized hydroxyapatite/polycaprolactone-graphene oxide based bioactive multifunctional coating on Ti alloy for bone implant applications. *Arab J Chem*. 2018;11(6):959–969. doi:10.1016/j.arabjc.2018.03.020
18. Ferraris S, Örlygsson G, Ng CH, Riccucci G, Spriano S. Chemical, physical, and mechanical characterization of chitosan coatings on a chemically pre-treated Ti6Al4V alloy. *Surf Coat Technol*. 2022;441:128571. doi:10.1016/j.surfcoat.2022.128571
19. Dvorakova J, Wiesnerova L, Chocholata P, et al. Human cells with osteogenic potential in bone tissue research. *Biomed Eng Online*. 2023;22(1):33. doi:10.1186/s12938-023-01096-w
20. Abdulwahab M, Khan AA, Abdallah SH, et al. Arylated gold nanoparticles have no effect on the adipogenic differentiation of MG-63 cells nor regulate any key signaling pathway during the differentiation. *BMC Res Notes*. 2021;14(1):192. doi:10.1186/s13104-021-05594-9

21. Niu M, Feng X, Zhou L. The role of the ERK1/2 pathway in simvastatin-loaded nanomicelles and simvastatin in regulating the osteogenic effect in MG63 cells. *Int J Nanomedicine*. 2018;13:8165–8178. doi:10.2147/IJN.S182998
22. Su J, Du Z, Xiao L, et al. Graphene oxide coated titanium surfaces with osteoimmunomodulatory role to enhance osteogenesis. *Mater Sci Eng C Mater Biol Appl*. 2020;113:110983. doi:10.1016/j.msec.2020.110983
23. Shin YC, Bae JH, Lee JH, et al. Enhanced osseointegration of dental implants with reduced graphene oxide coating. *Biomater Res*. 2022;26(1):11. doi:10.1186/s40824-022-00257-7
24. Ricci A, Cataldi A, Zara S, Gallorini M. Graphene-oxide-enriched biomaterials: A focus on osteo and chondroinductive properties and immunomodulation. *Materials (Basel)*. 2022;15(6):2229. doi:10.3390/ma15062229
25. Kim HD, Kim J, Koh RH, et al. Enhanced osteogenic commitment of human mesenchymal stem cells on polyethylene glycol-based cryogel with graphene oxide substrate. *ACS Biomater Sci Eng*. 2017;3(10):2470–2479. doi:10.1021/acsbiomaterials.7b00299
26. Xie H, Cao T, Franco-Obregón A, Rosa V. Graphene-induced osteogenic differentiation is mediated by the integrin/FAK axis. *Int J Mol Sci*. 2019;20(3):574. doi:10.3390/ijms20030574
27. Kumar P, Huo P, Zhang R, Liu B. Antibacterial properties of graphene-based nanomaterials. *Nanomaterials (Basel)*. 2019;9(5):737. doi:10.3390/nano9050737
28. Inchingolo AM, Malcangi G, Inchingolo AD, et al. Potential of graphene-functionalized titanium surfaces for dental implantology: Systematic review. *Coatings*. 2023;13(4):725. doi:10.3390/coatings13040725
29. Ke CL, Deng FS, Chuang CY, Lin CH. Antimicrobial actions and applications of chitosan. *Polymers (Basel)*. 2021;13(6):904. doi:10.3390/polym13060904
30. Georgopoulou A, Kaliva M, Vamvakaki M, Chatzinikolaidou M. Osteogenic potential of pre-osteoblastic cells on a chitosan-graft-polycaprolactone copolymer. *Materials (Basel)*. 2018;11(4):490. doi:10.3390/ma11040490
31. Alshiddi IF. Survival rate and clinico-radiographic parameters around narrow-diameter dental implants for fixed dental prostheses in the posterior regions:Aa systematic review. *Dent Med Probl*. 2023;60(2):345–353. doi:10.17219/dmp/140757
32. Erdilek D, Gümüştaş B, Güray Efes B. Digitalization era of dental education: A systematic review. *Dent Med Probl*. 2023;60(3):513–525. doi:10.17219/dmp/156804

Dental

and Medical Problems